Live fetuses were killed by an IP injection of sodium pentobarbitone solution and placed in alcohol for light fixation. Later they were skinned, dissected, the viscera examined and the sex recorded. A razor blade cut was made through the head along the frontal-parietal suture and the brain was examined. The carcasses were subsequently cleared in potassium hydroxide, stained with Alizarin red S to visualize the ossified skeleton and examined for skeletal variants and abnormalities.
Toxicokinetics: Not conducted.
Statistics:
Analysis of variance and William's test: Bodyweights and, if appropriate, bodyweight gains, Food consumption, Numbers of corpora lutea, implantation sites and live fetuses, Fetal bodyweight (by sex and on litter basis).
Kruskal-Wallis and Shirley's test: Pre-implantation loss, Post-implantation loss, Percentage of male foetuses (on litter basis).
Kruskal-Wallis and Fishers exact test: Fetal abnormalities.

Results:
For fertility studies:
In-life observations:
Mortality: There were six premature deaths during the course of the study.

Dose (mg/kg/d)	0	15	30	45
Animals Sacrificed	1/20 (#9)		2/20 (# 14, 57), Days 23 and 22 of pregnancy respectively. Macroscopic findings at necropsy for both animals included dark mesenteric lymph nodes and an abnormal red colored stomach.	1/24 (#74), macroscopic findings at necropsy consisted of an abnormal red colored stomach.

Dose (mg/kg/d)	0	15	30	45
Animals found dead.		1/20 (# 39)	1/20 (# 51)	

Sponsor stated that clinical signs and macroscopic findings at necropsy were consistent with gavage error.

Clinical signs: Reduced fecal production was noted in all animals including controls. The incidence was greater in the HD group where animals were observed with few or no feces. Incidences for LD and MD groups were lower and not dose-related. For animals sacrificed prematurely/found dead, clinical signs are as follows:
Animal # 74 (HD): reduced fecal production, partially closed eyes, hypoactivity and hunched posture.
Animal # 14 and 57 (MD) exhibiting a marked loss in bodyweight and a corresponding reduction in food consumption.
Animal # 9 (control): convulsion after gavage.
Animal #39(LD): red discharge from mouth and nose, labored breathing, vocalization, holding head up at an angle, unsteady gait, convulsion, found dead on GD 16. Sponsor stated that this animal struggled while being returned to the pen.
Animal # 51 (HD): Found dead.
Body weight: Statistically there is no significant difference between the weights of treated animals and that of control during dosing and after dosing. However, body weight gain showed a dose-dependent decrease during the dosing period (GD 6 to 18).
Body weight gain (kg)

Gestation Day	0 mg/kg/d	15 mg/kg/d	30 mg/kg/d	45 mg/kg/d
6-18	0.18	0.09* (50%↓)	0.20* (11%↑)	0.10** (44%↓)

*** p < 0.05; ** p < 0.01**

Food consumption: (g/rabbit/day)

Gestation Day	0 mg/kg/d	15 mg/kg/d	30 mg/kg/d	45 mg/kg/d
6-18	149	125	103** (31%↓)	92*** (38%↓)

*** p < 0.05; ** p < 0.01; *** p < 0.001**

Toxicokinetics: No data.

Terminal and necroscopic evaluations:

Female fertility

Dose (mg/kg/d)	0	15	30	45
Mean # of corpora lutea/female	10.6	11.1	11.3	10.9
Mean # of implantations/female	9.9	9.8	10.9	10.2
Mean pre-implantation loss	6.4	5.6	7.0	13.4
# of early embryo/fetal deaths	7	3	7	22
# of late embryo/fetal deaths	5	5	2	6
# of live fetuses/female	9.1	9.2	10.2	8.9
# of male fetuses	73	71	70	93
# of male fetuses	64	58	62	93
Mean litter weight	312.3	313.9	331.2	302.1
Mean fetal weight	34.5	35.3	32.9	34.3

Sponsor's statistical analysis showed no significant difference between treatment and control parameters.

***For embyrofetal development studies*:**

Terminal and necroscopic evaluations:

Offspring:

Fetal External Malformations: Unremarkable.

Fetal External Variations:

Dose (mg/kg/d)	0	15	30	45
Total # of fetuses examined	137	129	132	186
Total # of litters examined	15	14	13	21
Entire runted fetuses	0(0.0)	0(0.0)	3(2.4)*	3(1.3)*

*** $p < 0.05$**

Fetal Visceral Malformations: **No historical control data for Aortic arch-additional blood vessels.**

Dose (mg/kg/d)	0	15	30	45
Total # of fetuses examined	137	129	132	186
Total # of litters examined	15	14	13	21
Aortic arch: additional blood vessel	7(4.8)	17(12.4)*	23(17.7)**	66(37.9)***

*** $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$**

Fetal Skeletal Malformations: **Historical control data: mean (range)**

Dose (mg/kg/d)	0	15	30	45	HCD
Total # of fetuses examined	137	129	132	186	
Total # of litters examined	15	14	13	21	
Zygomatic arch & maxilla: partial fusion	0(	3(	8	5(	13.03

*** $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; HCD = Historical control data – mean (range)**

Fetal Skeletal Variations: **Historical control data: mean (range)**

Dose (mg/kg/d)	0	15	30	45	HCD
Total # of fetuses examined	137	129	132	186	Mean (Range)
Total # of litters examined	15	14	13	21	
Maxilla: incomplete ossification	18(12.0)	33(29.4)**	24(18.5)	58(35.4)***	13.6
Hyoid: incomplete ossification	50(31.7)	64(47.0)*	62(47.0)	92(50.2)*	0.84
5th Sternebrae: not ossified	18(12.7)	21(15.8)	45(34.5)***	57(29.8)***	32.7 (
Forelimb epiphyses: not ossified	29(19.3)	50(36.1)**	38(28.1)	58(31.7)*	20.82
Forelimb, one/more metacarpal: not ossified	14(9.3)	7(5.6)	17(12.6)*	27(16.2)*	2.12
Forelimb, one/more phalange: not ossified	2(1.9)	0(0.0)	5(4.1)	12(10.2)*	0.99
Forelimb, ≥ 1 phalange: incomplete ossific.	64(51.9)	45(33.9)	55(42.9)	115(64.1)**	24.25
Hindlimb, astragalus: not ossified	4(2.3)	2(1.4)	7(5.4)	17(10.1)*	0.29
Hindlimb, ≥ 1 phalange: incomplete ossific.	3(2.9)	0(0.0)	7(5.2)	14(9.6)*	6.33

*** $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; HCD = Historical control data – mean (range)**

Summary of Study Findings:
Groups of 20 pregnant rabbits were dosed with OGT 918 by oral gavage administration from Days 6 to 18 of pregnancy at dose levels of 0, 15, 30 and 45 mg/kg/day. The females were subjected to necropsy on Day 28 of pregnancy. There were 6 premature deaths. Sponsor stated that the demise of 2/20 (MD) and 1/24 (HD) females might be treatment-related. Sponsor attributed the rest of the deaths [1/29 (control), 1/20 (LD) and 1/20 (MD)] to dosing accident. Body weight gain was statistically significantly decreased in LD (50%) and HD (44%↓) females but increased in MD females relative to control. Food consumption decreased in a dose-dependent manner achieving statistical significance in MD (31%↓) and HD (38%↓) females.

Mean pre-implantation loss and early embryo/fetal deaths increased in a dose-dependent manner (not statistically significant). Fetal external variations revealed statistically significant increase in runted fetuses in MD and HD groups relative to control. Fetal visceral malformations (aortic arches with additional blood vessel) showed a dose-dependent and statistically significant increase in group mean incidence relative to control.

An increase in number and incidence of abnormalities or variants were noted in fetuses whose mothers received ≥ 15mg/kg/day; abnormalities included non- or incomplete ossification 5th sternebrae, metacarpals, epiphyses, phalanges of fore and hind limbs, partial fusion of the zygomatic arch and maxilla and runted fetuses. An additional blood vessel arising from the aortic arch was observed in all groups whose mothers received OGT 918. NOAEL for maternal toxicity could not be established because of maternal toxicity (decreased body weight gain) and mortality at doses ≥ 30 mg/kg/day. NOAEL for fetal/developmental toxicity could not be established because aortic arch anomaly (additional blood vessel) was observed at all dose levels, as well as incomplete/absent ossification of various bones. The fetal effects may be a function of maternal toxicity.

Study title: A Segment II teratogenic evaluation of SC-48334 in rabbits.

Key study findings:
- On GD 19, body weight gain decreased (not dose-dependently) by 33% in the HD fetuses relative to control.
- 1/112 fetuses were observed each with umbilical hernia and encephalomeningocele in the HD group.
- There was an increase in incidence of missing brachiocephalic in fetuses of treated dams relative to control. This observation in 40 fetuses/15 litters (HD) may be a little high compared to a historical control incidence of 40 fetuses/25 litters. Left subclavian branching variation increased in a dose-dependent manner and was 8-fold greater in HD fetuses relative to control. The incidence is 33 fetuses/15 litters compared to 28 fetuses/20 litters for historical control.
- Enlarged frontal fontanel showed a dose-dependent increase in fetuses of treated dams relative to control. The NOAEL for maternal toxicity was 10 mg/kg/d (0.6x maximum human dose of 100 mg based on mg/m^2) based on the slight decrease in body weight at the end of the dosing period. NOAEL for fetal/developmental toxicity was also 10 mg/kg/d because the missing brachiocephalic, enlarged frontal fontanel and frontal fontanel with reduced size observed in the LD group were within historical control range. The LD tested is 0.2x the human dose based on mg/m^2.

Study no.: PSA-89S-3489.
Volume #, and page #: Vol. 47, pg. 1.
Conducting laboratory and location: G. D. Searle & Co., 4901 Searle Parkway, Skokie, Illinois 60077.

Date of study initiation: March 27, 1989.
GLP compliance: Yes (USA).
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: OGT 918 lot # 88K023-302D, purity = 100%.
Formulation/vehicle: OGT 918 dissolved in distilled water.

Methods:
Species/strain: Rabbit/New Zealand White.
Doses employed: Total doses of 3, 10 and 30 mg/kg/d were administered as three equally divided doses.
Route of administration: Oral (gavage).
Study design: Total doses of 3, 10 and 30 mg/kg/d were administered as three equally divided doses to 16 female rabbits/group from gestation days (GDs) 6 to 18. The females were necropsied on GD 28.
Number/sex/group: 16 females/group.

Parameters and endpoints evaluated:
Clinical signs: Daily.
Mortality: Daily.
Body weight: Every 2 or 3 days.
Food consumption: Daily.
Terminal examination: On day 28 of gestation, the rabbits were sacrificed by an overdose of an euthanizing agent. Rabbits were caesarian sectioned and examined to obtain the numbers of corpora lutea, implantations, resorptions, and live or dead fetuses. All fetuses were individually weighed, examined for externally observable variations and/or malformations, and dissected for visceral variations and/or malformations as well as fetal sex. Subsequent to the visceral examination, all fetuses were stained with alizarin red and examined for skeletal variations and/or malformations.
Toxicokinetics: Not conducted.
Statistics: Sponsor described how the data would be analyzed but it appears no statistical analysis was conducted.
ANOVA and Levene's test for homogeneity of variance: Maternal body weights and body weights changes, and fetal body weights.
Kruskal-Wallis test: numbers of corpora lutea, implantations, resorptions, live or dead fetuses per litter, and pre-implantation losses; percent pre-implantation losses and percent post-implantation losses. If the Kruskal-Wallis test was significant at the 5% level, then the Mann-Whitney U test was used to compare control to each test article treated group.

Results:
***For fertility studies*:**
In-life observations:
Mortality: None.
Clinical signs: Sponsor stated that low food intake and/or not eating was observed in all groups including control but tended to increase in frequency with increasing dose (no data provided).
Body weight: (kg)

Dose (mg/kg/d)	0	3	10	30
GD 0	3.92	3.91	4.11	4.02
GD 19 (end of dosing)	3.98	3.88	4.08	4.06
Gain (GD 19 – GD 0)	**0.06**	-	-	**0.04**
GD 28 (Necropsy)	4.10	4.04	4.14	4.12
Gain (GD 28 – GD 0)	**0.18**	**0.13**	**0.03**	**0.10**

Data was not analyzed statistically

Food consumption: No data.
Toxicokinetics: No data.
Terminal and necroscopic evaluations:
Female fertility

Dose (mg/kg/d)	0	3	10	30
Total # of females	16	16	16	16
# live pregnant	15	14	14	16
# live not pregnant	1	2	2	0
# of corpora lutea/pregnant female	12.1	12.2	13.6	12.4
# of implantations/pregnant female	9.3	8.9	9.5	8.1
Pre-implantation loss/pregnant female	2.8	3.4	4.1	4.3
Pre-implantation loss (%)	21.6	27.3	29.5	33.3
# of resorptions/pregnant female	0.9	0.3	0.9	1.0
Post-implantation loss (%)	10.5	3.5	13.8	14.7
Total # of fetuses	125	120	121	114
# live	123	120	114	112
# dead	2	0	7	2
Live fetuses/pregnant female	8.2	8.6	8.1	7.0
Dead fetuses/pregnant female	0.1	0.0	0.5	0.1
Average wt. (g) of litter	37	37	34	36

Data not analyzed statistically; Historical control data submitted did not address these parameters.

***For embyrofetal development studies*:**
Terminal and necroscopic evaluations:
Offspring:

Dose (mg/kg/d)	0	3	10	30
FETUSES # examined	123(2)	120	114(7)	112(2)
# with malformations	1(1)	0	2(7)	3(2)
# of malformations	1(2)	0	2(7)	4 (2)
# with variations	3	4	2(4)	2(1)
# of variations	3	4	3(5)	3(2)
LITTERS # examined	14	14	14	15
# with fetal malformations	2	0	5	4
# with variations	3	3	3	2
Fetal External Malformations	**0**	**3**	**10**	**30**
Umbilical hernia	0	0	0	1
Encephalomeningocele	0	0	0	1
Enlarged eye bulge	0	0	1	0
Reduced eye bulge(s)	0(1)	0	1(7)	1(1)
Edema, lower side of face	0	0	0	0(1)
Edema, hind leg	0	0	0	1
Fetal External Variations	**0**	**3**	**10**	**30**
Eye(s) open	0	0	0(3)	0
Snub nose	0	0	0	1(1)
Foreleg flexion	0	0	0(2)	0(1)
Hematoma(s) along body surface	0	2	1	1

Number in parenthesis represent dead fetuses; Data not analyzed statistically; No historical control data for these parameters were not available.

Dose (mg/kg/d)	0	3	10	30	HCD
FETUSES # examined	123(2)	120	114(7)	112(2)	
# with malformations	7	14	12(1)	9	
# of malformations	9	15	12(1)	15	
# with variations	32	33	30(1)	64	
# of variations	32	39	36(1)	90	
LITTERS # examined	14	14	14	15	
# with fetal malformations	4	5	7	7	

# with variations	13	12	13	15	
Fetal Visceral Malformations	**0**	**3**	**10**	**30**	**HCD**
Left ventricle enlarged	0	0	0	1a	
Right ventricle enlarged	0	0	0	1a	
Ventricular septum missing	0	0	0(1)b	0	
Left atrium enlarged	0	0	1	0	
Pulmonary artery missing	0	0	0(1)b	0	
Pulmonary artery reduced in size	0	0	0	1a	
Aortic arch enlarged	0	0	0	1a	
Aneurysm on right carotid	0	0	0	1c	
Lung caudate lobe missing	0	10	1	4	
Gall bladder – doubled	0	0	0	1	
Spleen –r educed size	0	0	1	0	
Ureter(s) – distended	0	0	1	0	
R. ureter wrapped around inferior vena cava	0	0	0	1	
Brachiocephalic missing	19/13	25/12	21/13	40d/15	40/25
L. subclavian branching variation	4/13	4/12	7(1)e/13	33/15	28(2)/20
Large accessory vessel from L. common carotid	0	0	1	0	
Lung caudate lobe – reduced in size	0	4	1	2	
Ureters – slightly distended	4	3	4	7	
Liver – mottled in appearance	0	1	0	1c	
Spleen – pale color	0	0	0	1	
Kidneys – pale color	1	1	1	3f	
Ovaries – hemorrhagic	0	0	0	1c	
Umbilical arteries – hemorrhagic into surrounding tissue	0	0	0	1c	

Number in parenthesis represent dead fetuses; letters identify observations from same fetus; Data not analyzed statistically; HCD = Historical control data – fetuses/litter for some parameters was not available

Dose (mg/kg/d)	**0**	**3**	**10**	**30**	**HCD**
FETUSES # examined	123(2)	120	114(7)	112(2)	
# with malformations	44(2)	44	43(4)	42(2)	
# of malformations	52(3)	54	50(5)	52(2)	
# with variations	114(2)	110	108(7)	107(2)	
# of variations	269(14)	279	296(38)	278(11)	
LITTERS # examined	14	14	14	15	
# with fetal malformations	11	12	13	13	
# with variations	14	14	14	15	
Fetal Skeletal Malformations	**0**	**3**	**10**	**30**	**HCD**
Bone hole(s)	1	4	1	4	
Frontal fontanel enlarged	0(1)/44	2/44	3(3)/43	9(2)/42	6(6)/9
Frontal fontanel – reduced in size	10/44	15/44	18(1)/43	4/42	14/5
Interparietal split	0	6	1	2	
Floating thoracic rib	0	0	0	1	
Trace thoracic rib	0	0	0	1	
Fused ribs	1	0	2	1	
Thoracic centrum displaced	1	0	0	2	
Extra cervical vertebra	0	0	0	1	
Fetal Skeletal Variations	**0**	**3**	**10**	**30**	**HCD**
Frontal fontanel – irregularly shaped	0	2	1	0	
Hyoid centrum – incompletely ossified/unossified	61(2)e	52	79(5)	79(2)	
Hyoid process(es) – incompletely ossified/unossified	0	1	1(3)	0	
Sternebrae – incompletely ossified/unossified	1(1)	6	4(2)	10(2)	
Sternebrae – mishappen	6	4	9	1	
Cervical centrum – incompletely ossified/unossified	0	1	0	0	
Cervical centrum – misshapen	0	0	0	1	
Cervical 7th rib	0	0	0	1	
Thoracic centrum(s) – incompletely ossified/unossified	9	13	6	1	
Thoracic centrum(s) – misshapen	0	0	0	4	

Thoracic centrum(s) – split	0	0	1	1	
Lumbar vertebrae – missing.	0	0	0	1	
Proximal phalange(s) – unossified	9(2)	4	10(3)	13(2)	
Medial phalange(s) – unossified	41(2)	40	36(6)	48(2)	
Metacarpals – unossified	4(2)	17	16(4)	1	
Metatarsals – unossified	0(1)	0	0(1)	0	
Talus – incompletely ossified/unossified	0(1)	2	4(3)	0	
Calcaneus – incompletely ossified/unossified	0(1)	0	1	0	
Pubis – incompletely ossified/unossified	1	0	2(4)	7(1)	

Number in parenthesis represent dead fetuses; letters identify observations from same fetus; HCD = Historical control data – fetuses/litter; Data not analyzed statistically

Summary of Study Finding:

In the rabbit embryo-fetal toxicity or teratogenic study, OGT 918 at 0, 3, 10 or 30 mg/kg/day was given PO as three equal divided doses per day from Days 6-18 of gestation. On GD 19, body weight gain decreased (not dose-dependently) by 33% in LD and MD fetuses relative to control. There was a slight increase (dose-dependent) in pre-implantation loss/pregnant female and percent pre-implantation loss in treated females relative to control. Percent post implantation loss was relatively high in MD and HD treated females relative to control. Total number of fetuses as well as number of live fetuses decreased in a dose-dependent manner. Values for treated females were relatively less than that of control. Number of dead fetuses were increased by ~4-fold in MD females relative to control. Number of live fetuses/pregnant female decreased in a dose-dependent manner. Values for treated females were relatively less than that of control.

There was an increase in incidence of missing brachiocephalic in fetuses of treated dams relative to control. This observation in 40 fetuses/15 litters (HD) may be a little high compared to a historical control incidence of 40 fetuses/25 litters. Left subclavian branching variation increased in a dose-dependent manner and was 8-fold greater in HD fetuses relative to control. The incidence is 33 fetuses/15 litters compared to 28 fetuses/20 litters for historical control. Incidence of enlarged frontal fontanel showed a dose-dependent increase in fetuses of treated dams relative to control. However, the incidence of this observation in treated fetuses is lower than that of historical control. The NOAEL for maternal and developmental toxicity was 10 mg/kg/day (0.6x maximum human dose of 100 mg based on mg/m^2).

Study title: Oral (gavage) rat pre- and post-natal developmental toxicity study (Segment III).

Key study findings:

- 3 F0 females, 1/25 (MD – GD 22), 2/25 (HD – GDs 25 & 26) were sacrificed in extremis around the time of parturition. Sponsor stated that these animals had deteriorating clinical condition associated with extended parturition. None had surviving pups. Red vaginal discharge was observed in 14/25 HD females compared to 1/25 control females and was considered treatment-related.
- Body weight gain was decreased by 35% in HD F0 dams relative to control. This may be due to the statistically significant decrease (16%) in food consumption observed during GDs 15 to 20. Upon cessation of treatment (Day 1 – Day 21 post partum), body weight of the treated groups were similar to that of control.
- Food consumption was statistically significantly decreased by 16% in HD F0 dams during GDs 15 to 20 relative to control. Upon cessation of treatment, food consumption was still statistically significantly decreased by 17% in HD F0 dams (Day 1 – 7 post partum) and by 6% and 27% in MD and HD F0 dams (Day 7 – 14 post partum) respectively.

- Mean duration of gestation was slightly but statistically significantly increased in HD F0 dams relative to control. Mean number of pups born was statistically significantly decreased in HD F0 dams by 48% relative to control. Mean live birth index was statistically significantly decreased in MD (7%) and HD (25%) F0 dams relative to control. Mean cumulative survival index was also statistically significantly decreased in MD (12%) and HD (19%) F0 dams relative to control.
- Though not statistically significant, number of F1 pups found dead/killed premature was increased by 159% in LD and HD groups and by 263% in the MD group.
- Body weight gain of F1 pups was statistically significantly decreased (dose-dependently) in both males and females. In the HD group, the decrement was 18% and 17% for males and females pups respectively on Day 21 post partum.
- Body weight gain of F1 males was decreased by 3% (MD) and 9% (HD) relative to control. F1 females showed a dose-dependent decrease in body weight gain ranging from 6% (LD), 12% (MD) to 14% (HD) relative to control.
- Locomotor co-ordination (Rotarod test) in F1 males was statistically significantly decreased by 38% relative to control. Sponsor stated that this was considered to be associated with the lower offspring bodyweight in this group. MD and females (MD,HD) show decreased body weight but no effect in control.
- There was a slight but statistically significant delay in the onset of vaginal perforation in MD and HD F1 females relative to control.
- Growth and reproductive performance of F1 animals were not affected by maternal treatment.
- NOAEL for F1 generation growth, developmental, behavior and reproductive performance toxicity = 20 mg/kg. NOAEL for maternal toxicity is 60 mg/kg.

Study no.: WVC/014
Volume #, and page #: Vol. 47, pg. 49.
Conducting laboratory and location:

Date of study initiation: Febryary 5, 1999.
GLP compliance: Yes (UK).
QA reports: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: OGT 918 Batch # 60689-07; 99.3% pure.
Formulation/vehicle: A solution of OGT 918 in ultra high purity water.

Methods:
Species/strain: Rat/Sprague Dawley.
Doses employed: Total dose of 20, 60 and 180 mg/kg/d was administered as three equally divided doses.
Route of administration: Oral (gavage).
Study design:
Parental Females (F0): 25 females/group were mated with sexually mature males. The day on which mating was detected was designated Day 0 of pregnancy. The females were dosed three times daily, by oral gavage, from Day 6 of pregnancy to Day 20 post-partum (Day 0 post-partum was the day of completion of littering) inclusive. Females were allowed to rear their offspring to weaning on Day 21 post partum. The total dosage was administered as three equally divided doses, at least 6 hours apart. F0 were evaluated for clinical signs, body weight, food consumption, pregnancy duration/parturition and necropsied on Day 21 post-partum.

F1 Generation Animals: F1 were not dosed but were likely exposed in utero. It is not known if the drug is secreted into milk. 20 male and 20 female pups per group were randomly selected

for rearing to sexual maturity. Selection of the F1 generation was made approximately one week after the start of weaning of the F1 generation pups. F1 were evaluated for litter size, sex, clinical signs, mortalities, body weight, physical development, sensory/motor functions, learning, memory, ophthalmoscopy, Preyer reflex – hearing ability and sexual development.
F1 were reared untreated. At about 10 weeks of age, each female was paired with a male from the same dose group for up to 7 days. All mated females were subject to necropsy on Day 13 of pregnancy. Pregnancy status, number of corpora lutea, number and intrauterine position of implantations (classified as early resorptions, late resorptions or live embryos) were recorded. Males were killed approximately 2 weeks after completion of the mating period.
Number/sex/group: FO – 25 females/group; F1- 20 males and 20 females/group.
Parameters and endpoints evaluated:
Clinical signs: Daily.
Mortality: Twice daily.
Body weight: Female body weights were recorded daily from Day 5 of pregnancy until necropsy on Day 21 post-partum. Body weights recorded on Days 6, 7, 8, 9, 12, 15 and 20 of pregnancy and 1, 4, 7, 14 and 21 post-partum were reported.
Food consumption: Recorded and reported for Days 5 to 6, 6 to 9, 9 to 12, 12 to 15 and 15 to 20 of pregnancy and over Days 1 to 7 and 7 to 14 post-partum. Food consumption was not recorded during late lactation as the pups had started to eat diet.
Terminal examination:
F0: All parental females were killed on Day 21 post-partum at weaning of their litters. The uterus was examined and the number of implantation scars in each uterine horn recorded.
F1: A necropsy was conducted on all **pups** sacrificed or found dead during lactation and unselected pups after weaning. All mated F1 generation females were subject to necropsy on Day 13 of pregnancy. Pregnancy status, number of corpora lutea, number and intrauterine position of implantations (classified as early resorptions, late resorptions or live embryos) were recorded. F1 generation males were killed approximately 2 weeks after completion of the mating period.

Toxicokinetics: Not conducted.
Statistics:
ANOVA and William's test: Parental animal body weights, body weight gains, parental animal food consumption, number of corpora lutea, number of implantation sites, number of live embryos, number of pups born, pup body weights (by sex, on litter basis) on selected days and body weight gains, duration of gestation, duration of parturition, gestation index, E-maze run times, Rotarod data, Sexual development observations, Time course of mating.

Kruskal-Wallis and Shirley's test: Pre-implantation loss, post-implantation loss, litter survival indices, percentages of pups with developmental observations (on litter basis), percentage of male pups (on litter basis).

Fishers exact probability test (one-sided): Copulation index, pregnancy index, fertility index, E-maze decision data, ophthalmoscopy, auditory function (post weaning).

Results:
For Fertility studies
In-life observations: Dams
Mortality: 3 females, 1/25 (MD – GD 22) and 2/25 (HD – GDs 25 & 26) were killed in extremis around the time of parturition. Sponsor stated that these animals were sacrificed because of deterioration in clinical condition associated with extended parturition. None had surviving pups.

Clinical signs: Red/brown vaginal discharge was observed in 14/25 HD females compared to 1/25 control females and was considered treatment-related.

Body weight: (g)

Dose(mg/kg/d)	0	20	60	180
GD 6	228	225	226	226
GD 20	342	338	351	300***
Gain	114	113	125	74
Decrement	0	1	-	40
% decrement	0	0.9	-	35

*** p< 0.001

Dose(mg/kg/d)	0	20	60	180
Day 1 post-partum	228	225	226	226
Day 21 post-partum	288	286	288	282

No significant difference between control and treated groups

Food consumption: (g/rat/day)

Dose(mg/kg/d)	0	20	60	180
GD 5 – 6	19	19	18	19
GD 15 – 20	25	25	25	21*** (16%↓)

*** p< 0.001

Dose(mg/kg/d)	0	20	60	180
Day 1-7 post-partum	36	36	36	30***(17%)
Day 7-14 post-partum	55	54	52**(6%)	40***(27%)

** p< 0.01, *** p< 0.001

Toxicokinetics: No data.

Mating/Fertility data of FO

Dose(mg/kg/d)	0	20	60	180
Gestation index	100	100	100	83.3
Mean duration of gestation (days)	21.9	21.8	21.9	22.7***
Mean duration of parturition	Unremarkable			
Mean # of pups born	11.8	11.3	12.6	6.2***(48%)
Mean live birth index	93.9	92.3	87.0*(7%)	70.5**(25%)
Mean viability index	Unremarkable			
Mean cummulative survival index	90.8	84.9	79.9*(12%)	73.2**(19%)
Sex ratio at birth	Unremarkable			

* p< 0.05,** p< 0.01, *** p< 0.001

Terminal and necroscopic evaluations – Dams: Unremarkable.

For embyrofetal development studies:

In-life observations: Offspring – Pups

F1 Fetal Parameters:

Dose(mg/kg/d)	0	20	60	180
Total # of litters	24	25	25	23
# of pups born	283	282	315	149
# found dead/killed prematurely	27	43(159%)	71(263%)	43(159%)
Missing/presumed cannibalized	0	3	3	15

Clinical signs: Clinical signs were those of poor maternal care (scattered, not cleaned, cold, not fed). This may account for the decreased pup weight at all doses.

Body weight: Pups (g)

Males Dose(mg/kg/d)	0	20	60	180
Day 0 post-partum	6.7	6.6	6.6	6.0***
Day 21 post-partum	49.6	48.0	46.8*	41.0***
Gain	42.9	41.4(3%↓)	40.2*(6%↓)	35***(18%↓)

* p< 0.05, *** p< 0.001

Females Dose(mg/kg/d)	0	20	60	180
Day 0 post-partum	6.3	6.3	6.3	6.0*
Day 21 post-partum	46.8	45.7	44.5	39.7
Gain	40.5	39.4(3%↓)	38.2*(6%↓)	33.7***(17%↓)

* p< 0.05, *** p< 0.001

Terminal and necroscopic evaluations – Pups:

Dose(mg/kg/d)	0	20	60	180
Total # of litters	24	25	25	23
# of pups born	283	282	315	149
# found dead/killed prematurely	27	43	71	43
Missing/presumed cannibalized	0	3	3	15
# of culled pups	69	55	61	7
# of pups killed post weaning	147	141	140	51
Findings: Dead/moribund/sacrificed pups				
Partially bitten/cannibalized	3	1	1	5
Autolyzed	2	1	3	2
Lungs not inflatted	7	8	14	10
Stomach – no milk	14	32	49	24
Culled pups	0	0	1	0
Pups post-weaning	0	1	0	0

For peri-postnatal development studies: F1 Generation

In-life observations: Offspring:

Mortality: 1/5 males, an offspring of a HD F0 treated female was killed in extremis due to mouth lesions and overgrown lower teeth. Sponsor did not consider this treatment-related.

Clinical signs: There were no treatment-related clinical signs that bore relationship to F0 maternal treatment.

Body weight: (g), n = 20

F1 Generation Males	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
Week 1	73	71	68*	59***(19%)
Week 10	399	401	385*	356***(11%)
Gain	326	330	317	297
% Decrement	0	-	3	9

* p< 0.05, *** p< 0.001

F1 Generation Females, n = 20

Pre-mating	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
Week 1	68	66	64	57
Week 7	225	225	227	211**(6%)
Gain	157	159	163	154
% Decrement	0	-	-	2

* p< 0.05, ** p< 0.01, *** p< 0.001

F1 Generation Females; n = 16(0), 17 (LD), 18 (MD), 17 (HD).

Day of pregnancy	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
0	228	229	235	217*(5%)
13	294	291	293	274***(7%)
Gain	66	62	58	57
% Decrement	0	6	12	14

* p< 0.05, ** p< 0.01, *** p< 0.001

Food consumption: No data.

E-maze Learning Test – F1 Generation: There was no effect of F0 maternal treatment on the learning and memory of male or female pups in any of the groups receiving OGT 918. Run times, correct turn decisions and 24 hour memory retention was similar in all groups.

Ophthalmoscopy – F1 Generation: There were no changes in ophthalmoscopy findings in any group that could be related to maternal treatment.

Auditory Acuity – F1 Generation: There was no effect of maternal treatment on the auditory acuity, in any group, as assessed by the Preyer response with all groups recording 100%.

Locomotor co-ordination – F1 generation:

F1 Generation Males

Rotarod Test data (sec)	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
Mean of 5 Runs	144	117	130	90*(38%)

*** p< 0.05**

F1 Generation Females

Rotarod Test data (sec)	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
Mean of 5 Runs	158 ± 66	151 ± 84	134 ± 71	114 ± 74

SD included to demonstrate non statistical significance

Sexual Development Observations – F1 Generation: n = 20

Dose (mg/kg/d)	Day Preputial separation was observed	Day Vaginal perforation was observed
0	46	34
20	45	35
60	45	36**
180	46	37***

**** p< 0.01, *** p< 0.001**

Mating and Fertility Data – F1 Generation

Dose (mg/kg/d)	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
Time course of mating (females)	Unremarkable			
# mated	18/20	18/20	18/20	20/20
# fertile	16/20	17/20	18/20	17/20
Copulation index	90%	90%	90%	100%
Fertility index	88.9%	94.4%	100%	85%

NS = no statistical significance

Terminal and necroscopic evaluations – F1 Dams:

Dose (mg/kg/d)	0 mg/kg/d	20 mg/kg/d	60 mg/kg/d	180 mg/kg/d
# of females with implantations at scheduled kill	16	17	18	17
Mean # of corpora lutea/female	16.4	16.3	15.8	15.2
Mean # of implantations/female	15.7	15.5	15.3	14.2
Mean % pre-implantation loss	4.2	5.3	3.5	6.2
# of early embryo/fetal deaths	17	9	13	11
Mean post-implantation loss	6.9	3.2	4.7	4.5
Mean # of live embryos/female	14.6	14.9	14.6	13.5
Mean # of implantations	93.1	96.8	95.3	95.5

NS = no statistical significance

Summary of individual study findings:

Groups of 25 female rats were dosed by oral garage at dose levels of 0, 20, 60 and 180 mg/kg/day to investigate the effects of OGT 918 on embryonic, fetal and post-natal development of the rat following administration to mated females from Day 6 of pregnancy, throughout lactation to Day 20 post-partum. The females were necropsied on Day 21 of lactation. Approximately one week after the start of weaning of the F1 offspring, 20 male and 20 female offspring were randomly selected. The F1 generation was allowed to mature, untreated and the effects on growth, development, behavior and reproductive performance were assessed.

3 F0 females, 1/25 (MD – GD 22), 2/25 (HD – GDs 25 & 26) were killed in extremis around the time of parturition. All three showed deterioration in condition and had no surviving offspring. Red vaginal discharge was observed in 14/25 HD females compared to 1/25 control females and was considered treatment-related. Body weight gain was decreased by 35% in HD F0 dams relative to control. This may be due to the statistically significant decrease (16%) in food consumption observed during GDs 15 to 20. Upon cessation of treatment (Day 1 – Day 21 post partum), body weight of the treated groups were similar to that of control. Food consumption was statistically significantly decreased by 16% in HD F0 dams during GDs 15 to 20 relative to control. Upon cessation of treatment, food consumption was still statistically significantly decreased by 17% in HD F0 dams (Day 1 – 7 post partum) and by 6% and 27% in MD and HD F0 dams (Day 7 – 14 post partum) respectively.

Mean duration of gestation was slightly but statistically significantly increased in HD F0 dams relative to control. Mean number of pups born was statistically significantly decreased in HD F0 dams by 48% relative to control. Meal live birth index was statistically significantly decreased in MD (7%) and HD (25%) F0 dams relative to control. Mean cummulative survival index was also statistically significantly decreased in MD (12%) and HD (19%) F0 dams relative to control. Though not statistically significant, the number of F1 pups found dead/killed premature was increased by 159% in LD and HD groups and by 263% in the MD group. Body weight gain of F1 pups was statistically significantly decreased (dose-dependently) in both males and females. In the HD group, the decrement was 18% and 17% for males and females pups respectively on Day 21 post partum. Body weight gain of F1 males was decreased by 3% (MD) and 9% (HD) relative to control. F1 females showed a dose-dependent decrease in body weight gain ranging from 6% (LD), 12% (MD) to 14% (HD) relative to control.

Locomotor co-ordination (Rotarod test) in F1 males was statistically significantly decreased by 38% relative to control. Sponsor stated that this was considered to be associated with the lower offspring bodyweight in this group. There was a slight but statistically significant delay in the onset of vaginal perforation in MD and HD F1 females relative to control. Growth and reproductive performance of F1 animals were not affected by maternal treatment. NOAEL for F1 generation growth, developmental, behavior and reproductive performance toxicity was considered to be 20 mg/kg (0.6x maximum dose based on mg/m^2). NOAEL for maternal toxicity was 60 mg/kg (2x maximum dose based on mg/m^2).

Reproductive and developmental toxicology summary:

In the male rat fertility studies, OGT 918 affected normal morphology (headless sperms, sperms with reduced hooks & miscellaneous abnormalities) and motility (VAP) of sperm (≥ 20 mg/kg/d), which was consistent with the observed reduction in fertility. Reversibility of these parameters was demonstrated on cessation of treatment (6 or 13 week recovery). In further studies on male fertility, rats were dosed and mated with untreated females. The effects on male fertility and sperm parameters were confirmed. Treatment resulted in an increase in the number of unfertilised and fragmented ova, but those that were fertilised proceeded to develop normally. When males were mated after a 6-week treatment-free period, pregnancy parameters were within normal ranges. Sperm morphology and motility was similar to that of control animals after a 13-week treatment-free period. NOAEL could not be established because of the decreased sperm concentration, decreased number of normal sperms, increased headless sperms and increased sperms with reduced hooks in LD males. However, the LD tested is 0.6x the human dose based on mg/m^2.

When OGT 918 was administered to female rats prior to mating through GD 17, fetotoxic effects were observed at exposures associated with maternal toxicity. The NOAEL for maternal toxicity

was 60 mg/kg/day (2X human exposure based on mg/m2) based on a statistically significant decrease in body weight (10%) and body weight gain (-29%) at 180 mg/kg/day. Post-implantation loss was observed at doses ≥ 60 mg/kg/day. The NOAEL for fetal developmental toxicity was 20 mg/kg/day (<1X human exposure based on mg/m^2) based on a significant increase in early embryo fetal deaths at ≥ 60 mg/kg/day (24 vs. 10 in control). Several fetal findings are observed associated with maternally toxic exposures (>60 mg/kg/day). Increased placental weight, in addition to the following visceral malformations: absent innominate artery, misshapen ventricles, reduced lung size, increased kidney pelvic cavitation and dilated ureters are found in fetuses from dams given 180 mg/kg/day. Only the absent innominate artery incidence exceeds control and historical control mean and range at 180 mg/kg/day; at a dose associated with maternal toxicity. Decreased fetal weight was observed at 180 mg/kg/day. Several skeletal malformations are observed at doses ≥ 60 mg/kg/day including: wavy ribs, malformed scapula, misshapen sternum, misshapen vertebrae most of these findings are within the historical control range. Skeletal variations consisting of incomplete ossification of: occipital, thoracic vertebra, sternebra (1-6), pubis, metacarpals (forelimb), phalanges (forelimb) and metatarsals (fore and hind limb) bones is observed at doses ≥ 60 mg/kg/day. However the incidences of these findings may exceed control and historical mean but are within the historical control range.

In rabbits dosed with 15, 30, 45 mg/kg/day during organogenesis (GD 6-18) maternal toxicity was evident at 15 mg/kg/day with a -50% decrease in body weight gain and mortality (2/20 GD 22-23) at 30 mg/kg/day. It is unclear if the premature deaths were specifically related to parturition as in the rat although they were considered treatment related. Macroscopic exam of those dams consisted of dark mesenteric lymph nodes and abnormal red stomach on GD 22 and 23. Statistically significant, dose dependent increase in additional aortic arch blood vessels at all dose levels and incomplete ossification of maxilla, hyoid and forelimb epiphyses (not ossified) at 15 mg/kg/day. The fetal findings at 15 mg/kg/day (<1X human therapeutic exposure) occur at exposures associated with maternal toxicity. An earlier rabbit Segment II study using 3, 10, 30 mg/kg/day during GD 6-18 conducted by Searle (the sponsor at the time) did not show maternal mortality although body weight gain decreased by 33% at 30 mg/kg/day. The fetal/litter incidence of missing brachiocephalic artery increased with dose but remained below the historical control mean (range not provided). The presence of a left subclavian branching variation increased by fetal incidence at ≥ 10 mg/kg/day but remained below historical control fetal/litter mean incidence. Skeletal variations including: misshapen thoracic centrum, unossified medial phalange and pubis were increased at 30 mg/kg/day whereas incomplete ossification of the hyoid centrum occurred dose dependently at 10 mg/kg/day and incomplete sternebra at 3 mg/kg/day (not dose dependently). Historical control data is not provided, however the findings occur in general at doses associated with maternal toxicity and the incomplete ossification which occur at lower doses are consistent with the previous study.

In a Segment III study where rats were given 20, 60, 180 mg/kg/day during organogenesis through lactation (GD6- PPD 20) dystocia and delayed parturition were observed. Three females were sacrificed in extremis Gestation Day 22, 25, 26, 1/25 in MD and 2/25 in HD groups as a function of deteriorating clinical condition associated with extended parturition. None had surviving pups. A 35% decrease in body weight gain was observed at the HD. An increased gestational duration (22.7 vs. 21.9 days control), a 48% decrease in births and 25% decrease in live births were observed at HD. At >60 mg/kg/day the mean cumulative pup survival index decreases dose dependently by 12-19% and pup body weight gain decreases dose dependently by 6-18%. Drug treated groups had increased numbers of dead or sacrificed in extremis pups by 159% at >20 mg/kg/day. Most of these animals were found with uninflated lungs and absence of milk in the stomach at necropsy. Locomotor co-ordination assessed by

mean rotarod test data (n=5 runs), shows a significant decrease in HD males. The sponsor attributes this to decreased bodyweight. However pup body weights are similarly reduced in females and MD rats which did not show adverse effects in locomotor co-ordination. A statistically significant delay (2-3 days) in the onset of vaginal perforation in pups from groups dosed ≥ 60 mg/kg/day. Mating and fertility parameters of the crossed FI generation were unremarkable.

Study title: Oral (Gavage) Juvenile Developmental toxicity study

Key study findings:

- Mortality was observed at all dose levels: 2/80 (0 mg/kg), 1/80 (LD-20 mg/kg), 5/80 (MD-60 mg/kg) and 3/80 (HD-180 mg/kg). The causes of demise of these animals were not disclosed and it is not apparent from the individual histopathology data. Sponsor stated that they were not treatment related.
- Clinical signs of head tilting (left) was observed in all dose groups including control. The incidence did not show any dose-response. This could be due to neurotoxicity. Dose-dependent increase in incidence of distended abdomen was observed at doses ≥ 60 mg/kg/d and it is treatment related. Ridges on whole tail was observed at doses ≥ 20 mg/kg/d. Incidence did not show dose-dependency. Clinical signs observed in animals sacrificed in extremis included hypoactivity, shallow/labored breathing, hunched posture, pale/cold extremities, piloerection, unsteady gait (1 male – MD), and tremors (1/40 female – MD).
- Body weight gain was statistically significantly decreased in all treated animals relative to control by pre-weaning day 21. There were no significant differences in body weight/body weight gain between control and treated groups during the post weaning (weeks 3-10) and recovery periods.
- Food consumption was slightly but statistically significantly decreased during weeks 1-2 in both Day 35 and Day 70 sacrificed animals. For the Day 70 sacrificed animals, food consumption increased significantly in all treated males and HD females relative to control. At the end of the recovery period, there was no significant difference between control and treated animals.
- Lens suture lines was statistically significantly increased in LD males relative to control. Since this observation is not dose-dependent, it may be an incidental finding.
- For the blood samples taken on Day 21, hemoglobin, PCV, MCV and MCH were slightly but statistically significantly decreased in treated animals relative to control. WBC and lymphocytes were significantly increased in treated males relative to control. Blood samples taken on Day 70 showed significant decreases in RBC (HD-M) and MCHC (MD & HD-F). WBC, lymphocytes and monocytes were significantly increased in MD and HD animals relative to control. Basophils were slightly but significantly increased in MD and HD males only.
- Creatinine was slightly but significantly decreased in MD and HD females. Glucose was significantly increased in MD males and HD males and females. Alkaline phosphatase was significantly decreased in all treated males but not in a dose dependent manner. ALT was slightly but significantly increased in MD and HD females. Albumin was significantly decreased in all treated animals. Globulin was slightly but significantly decreased in all treated females and so was the A/G ratio. Total protein was slightly but significantly decreased in HD males. Cholesterol was significantly increased in all treated females relative to control. Triglycerides were slightly but significantly increased in all treated males and in MD and HD females. Ca was slightly but significantly increased MD and HD animals relative to control. Phosphorus was slightly but significantly increased in MD and HD females; Na was slightly but significantly decreased in MD and HD females, whereas, K was

slightly but significantly increased in all treated females and in MD and HD males relative to control.

- Absolute weight of the brain was slightly but statistically significantly decreased in treated females relative to control. This may be due to the slight decrease in body weight. Absolute weight of the heart was slightly but statistically significantly increased in HD females. Relative weight of the heart was slightly but statistically significantly increased in MD females and in HD males (myocarditis) and females. Since there is no correlative heart histopathology in females to explain the increased relative weight, it may be due to the slight decrease in body weight. Absolute weight of the liver was significantly increased in MD and HD females. Relative liver weight was significantly increased in all treated animals. The increased relative liver weight may be due to the slight increase in ALT (MD and HD females), the liver inflammation (all treated males) and bile duct proliferation (HD males) observed or due to the slight decrease in body weight. Absolute weight of the ovaries was slightly but significantly increased in HD females. Both absolute and relative weights of the kidney were slightly but significantly increased in MD and HD females. However, there is no correlative histopathology in females to explain the increased relative kidney weight. In males, kidney weight was not significantly increased but there is hydronephrosis and basophilic tubules (non reversible). Both weights of the thyroid were slightly but significantly increased in all treated females and in HD males. Since there is no correlative histopathology, these increments may be due to the slight decrease in body weight. Relative weight of the thymus was slightly but significantly increased in all treated males with no correlative histopathology. Relative weight of the testes was also slightly but significantly increased in HD males. This correlates with the degeneration of the germinal epithelium observed.
- Sperm concentration was significantly decreased in a dose-dependent manner at Day 70. With regards to sperm morphology, number of normal sperms was significantly decreased whereas number of headless sperms was significantly increased in all treated males relative to control. Number of sperms with reduced hook was significantly increased in a dose-dependent manner. Miscellaneous abnormalities were also significantly increased in all treated males relative to control. Average number of spermatids was also significantly decreased in all treated males relative to control. At the end of the 10 week recovery period, all sperm parameters were similar to those of control except for the significant increase in miscellaneous sperm abnormalities in all treated males relative to control.
- OGT 918 had no effect on physical development, learning, locomotor activity, auditory function, righting reflex, air righting reflex, vision and hindlimb tactile placing of the F1 pups. There was no effect of treatment in any group on the home cage, open field and removal from home cage observations.
- Marked diffuse brain vacuolation, marked sciatic nerve vacuolation and slight to marked tibial nerve vacuolation was observed in weanlings dosed ≥ 20 mg/kg/d.
- There was a delay in the day balanopreputial separation occurred in males and the onset of vaginal perforation in females in all groups treated with OGT 918; statistical significance was achieved in HD males and all treated females.
- The target organs of toxicity included the epididymides (↓ sperms), heart (chronic myocarditis), kidney (hydronephrosis, basophilic tubules), liver (inflammation, bile duct proliferation), testes (degeneration of germinal epithelium), brain, sciatic and tibial nerves (diffuse vacuolation) and submandibular lymph node (erythrophagia, lymphoid hyperplasia).
- NOAEL could not be established because of decreased sperm concentration, altered sperm morphology, kidney effects (hydronephrosis, basophilic tubules), liver inflammation and chronic myocarditis observed at the LD. Moreover, mortality occurred at all dose levels including control and it not clear if this is drug-related or not.

Study no: WVC0024
Volume #, and page #: Vol. 9, pg. 1.
Conducting laboratory and location:

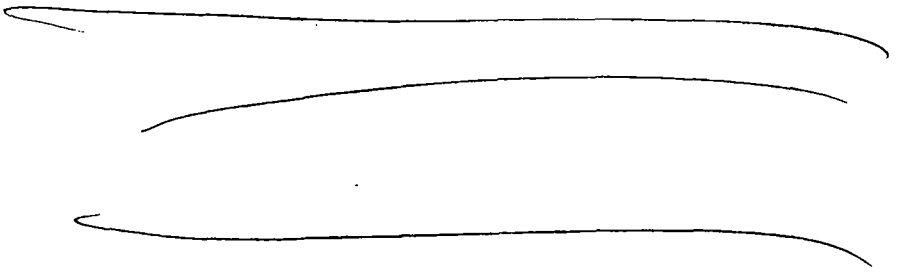
Date of study initiation: March 3, 2000.
GLP compliance: Yes (U. K.).
QA report: Yes (X) no ( )
Drug, lot #, radiolabel, and % purity: OGT 918 Lot # 60689-07, 99.3% pure.
Formulation/vehicle: A solution of OGT 918 in ultra high purity water.

Methods (unique aspects):
Dosing: The F1 pups were dosed orally by gavage once daily from Days 12 to 21 postpartum, inclusive. From Day 22 postpartum onwards the animals were dosed TID for approximately 7 weeks. The total doses administered were 20, 60 and 180 mg/kg/d.
Species/strain: Rats/Sprague Dawley (SD)
#/sex/group or time point (main study): 40 male and female F1 pups/group.
Satellite groups used for toxicokinetics or recovery: 10/sex/group for TK analysis.
Age: 12 days at start of dosing.
Weight: 27 g (M), 25 g (F) F1 generation.
Doses in administered units: 20, 60 and 180 mg/kg/d.
Route, form, volume, and infusion rate: Oral (gavage), solution, 10 ml/kg.

Observations and times:
Clinical signs: Daily observation for changes in behavior and appearance.
Body weights: Pups were weighed individually on Days 1, 4 and 11 postpartum. Sponsor stated that since dosing commenced on Day 12 postpartum, body weights on Days 1, 4 and 11 were not presented in this report. From Days 12 to 21 postpartum, F1 generation were weighed daily. Thereafter, body weights were recorded twice weekly.
Food consumption: Weekly.
Ophthalmoscopy: Eyes of each pup was examined at 42 days of age.
EKG: Not conducted.
Hematology: On Day 21 blood samples were collected from non-fasted animals (5/sex/ group). On Day 70 blood samples were collected from fasted animals (10/sex/group) for blood chemistry analysis.
Clinical chemistry: On Day 21 blood samples were collected from non-fasted animals (5/sex/ group). On Day 70 blood samples were collected from fasted animals (10/sex/group) for blood chemistry analysis.
Urinalysis: Not conducted.
Gross pathology: Organs/Tissues collected for gross pathology is indicated in the list of addendum.
Organs weighed: Organs weighed are indicated in the list of addendum.
Histopathology: Tissues examined are indicated in the list of addendum.
Toxicokinetics: Blood samples were taken (approximately 30 minutes after dosing) at necropsy on Days 21 35, and 70 from ten males and ten females per group for proof of absorption of test article.
Other:
Pup Development: Number of pups in each litter exhibiting the following characteristics was recorded:

1. Ears open (examined daily until occurrence and reported as the percentage of pups in the litter with ears open on Day 4).

2. Eyes open (examined daily until occurrence and reported as the percentage of pups in the litter with eyes open on Day 15).
3. Static righting reflex - the pup was placed on its back and the reflex regarded as present when the pup turned over onto all four paws within 10 seconds (examined on Day 5).
4. Startle response - a sharp noise was made at a distance approximately 5 cm behind the pup and the reflex regarded as present if the pup flinched (examined on Day 15 of age - not including non-selected pups).
5. Pupillary light reflex - a pin point light was shone into both eyes of each pup and the reflex regarded as present if the pupil responded by contraction (examined on Day 21 of age - before selected necropsy).



1. Correct or incorrect *mm* in the direction of the exit platform when the pup passed the decision point (DP).
2. Time taken to complete the run.
3. Any abnormal locomotor activity or behavior.

The learning potential of the pups was assessed in session 1 by the decrease in time taken to complete the maze and in the number of correct turns.

Memory retention over seven days was assessed in session 2 by the direction of the turn in the first run compared with the correct direction of the exit platform in session 1.

Re-learning potential was assessed in session 2 by the times taken to complete the maze and by the number of correct turns when the exit platform was located in the other arm of the maze.

Motor Activity: At approximately 29 to 34 days post-partum, motor activity was tested individually using the [redacted] fitted with photobeam mountings to detect activity [redacted]

The activity system is fully automated and was located in a separate room equipped with a white noise generator to control for extraneous background noise. The maze consisted of several interconnected alleyways which form a "figure 8" and elicits moderately high levels of spontaneous motor activity, thus detection of both increases and decreases in activity are possible. Each pup was tested in the maze for a period of approximately 20 minutes.

Locomotor Activity: At approximately 28 days of age, locomotor activity was assessed using a rotarod.

Auditory function: At approximately 36 days of age, the auditory function of each pup was assessed using the Preyer reflex.

Sexual development observations: All selected males were examined daily for balanopreputial separation from the time that the first male was 35 days postpartum until all the males showed balanopreputial separation or the last male was 50 days post-partum.

All selected females were visually examined daily for vaginal perforation from the time that each female was 28 days post-partum until all the females showed vaginal perforation or the last female was 40 days post-partum.

Functional Observation Battery: Selected pups from all dose groups were tested on Days 21 and 60 post-partum. The same trained observer evaluated all the animals. The observations started with the animals in the home cage, continued as the animals were removed from the home cage, placed in the standard arena and then observed in this arena, finishing with the most interactive ('other') tests.

Home Cage:

a). Descriptive assessment of postural and gait abnormalities. Abnormalities were scored as follows: 0 = absent 1 = slight 2 = moderate 3 = extreme

b). Presence of convulsions, tremors or abnormal motor movements. These were described and then scored as for posture and gait.

c). Assessment from cage tray-liners of disorders of urination and defecation, including polyuria and diarrhea.

On Removal from Home Cage:

a). Reactivity on removal from the cage was scored as follows:
0 = no response, 1 = normal avoidance reaction,
2 = exaggerated avoidance, struggling once held or vocalization,
3 = attempting to bite handler.

Performance in an Open Field:

A circular solid-bottomed arena, sufficiently high to allow the animals to stand on their back legs, was used and the following were assessed over a two minute period.

a). The arousal level or state of alertness of the animal, once settled and unperturbed in the open field, was scored as follows: 0 = comatose, 1 = hypoactive, 2 = normally active, 3 = hyperactive.

b). Descriptive assessment of postural and gait abnormalities. Abnormalities were scored as follows: 0 = absent, 1 = slight, 2 = moderate, 3 = extreme.

c). Presence of convulsions, tremors or abnormal motor movements. These were described and then scored as for posture and gait.

d). Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypes), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose or mouth and any other observations.

e). Assessment of disorders of urination and defecation including polyuria and diarrhea.

f). Alterations in the rate and ease of breathing were recorded.

g). Excessive or spontaneous vocalization was noted.

Other Tests

a). Vision was assessed utilizing the visual placing response. Each animal was held by the base of its tail above an inclined grid. The animal was moved towards the grid and if it outstretched its forelimbs to touch the grid before it actually reached the grid, it received a score of 0. If it failed to outstretch its forelimbs to the grid, until it had touched the grid, then it received a score of 1. The procedure was performed once for each animal.

b). Hindlimb tactile placing response was assessed by bringing the animal towards a suitable surface with a well defined edge (i.e. table or cage) such that its ventral abdomen was brought into contact with a right angle comer, and its hindlimbs were free to scrabble for grip.

c). Righting reflex was tested as described by Tupper D.E and Wallace R.B (1980). "Utility of the neurological examination in rats", Acta Neurobiologica Experimentia, 40, pp 999-1003.

The animal was put on its back whereupon it should have turned over immediately. It was then held in the lower back, its body was tilted whereupon its head should have moved in the opposite direction. Each procedure was carried out twice. If responses were prompt a score of 0 was given, if absent a score of 2 was given and if sluggish a score of 1 was given. The maximum score for each occasion was therefore 8, the minimum 0.

d). Air righting reflex was only tested on those animals exhibiting some righting reflex in the above test (Tupper and Wallace). Any animal excluded for this reason was given the maximum 6 points. The animal was held on its back in the air approximately 30 cm above a bed of sawdust. The normal animal, when released, landed squarely on the feet and a score of 0 was given. If it landed on its side, a score of 1 was given and if it landed on its back, a score of 2 points was given. The test was performed 3 times for each animal.

Sperm analysis: For male animals sacrificed on Day 70 of age and those sacrificed after the ten week recovery period, the testes and epididymis were removed and the testes and left epididymis were weighed. The right cauda epididymis from all males was used for sperm examination. Samples of sperm were collected for examination.

Results:

Mortality: Group 1 = 0 mg/kg/d, 2 = 20 mg/kg/d, 3 = 60 mg/kg/d, 4 = 180 mg/kg/d. Following commencement of dosing on Day 12 of age, one control animal was found dead and 10 others were sacrificed.

Group	Sex	Number	Day of death
1	Female	023 #	15 post-dose
1	Male	074 $	14 found dead
2	Male	171 #	13 post-dose
3	Female	386	19 pre-dose
3	Male	363	22 post-dose
3	Female	475	53 post-dose
3	Female	476	42 post-dose
3	Male	484	14 post-dose
4	Male	332	16 pre-dose
4	Female	337	17 pre-dose
4	Male	632	13 pre-dose

= Animals replaced on study by other offspring on Day 11 of age. No data was presented for these early decedent replaced animals.

$ = Animals replaced on study by other offspring on Day 14 of age (not dosed on Days 12 and 13. No data was presented for the early decedent replaced animal.

Clinical signs: Signs observed in animals that survived till necropsy.

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Head tilting - left	2/40	2/40	1/40	3/40	4/40	3/40	2/40	1/40
Distended abdomen	0/40	0/40	0/40	0/40	1/40	1/40	20/40	20/40
Ridges on whole tail	0/40	0/40	0/40	4/40	27/40	30/40	23/40	24/40

Clinical signs observed in animals sacrificed in extremis included hypoactivity, shallow/labored breathing, hunched posture, pale/cold extremities, pilorerction, unsteady gait (1 male – MD), and tremors (1 female – MD).

Body weights: (g)

F1 Generation Pre-weaning weights (g)

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Pre-weaning-Day 12	27	25	26	25	26	25	27	26
Pre-weaning-Day 21	51	47	48*	45^	46***	44^^^	45***	42^^^
Mean body wt. gain	24	22	22***	20^^^	20***	19^^^	18***	16^^^

* p < 0.05, *** p < 0.001 Shirley' test; ^ p < 0.05, ^^^ p < 0.001, Williams test

F1 Generation Post-weaning weights (g)

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Week 3	51	46	48	45	48	45	44	41
Week 10	315	213	304	216	307	215	304	211
Mean body wt. gain	264	168	258	172	259	170	260	170

No significant difference between control and treated groups

F1 Generation Recovery body weights (g): there was no significant difference between control and treated groups.

Food consumption:

F1 Generation – Day 35 sacrifice (g/animal/d)

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Weeks 1 - 2	12	11	12	11	11	10*	10**	10**
Weeks 2 - 3	15	14	14	14	15	13	14	13

* p<0.05, ** p<0.01, Shirley's test

F1 Generation – Day 70 sacrifice (g/animal/d)

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Weeks 1 - 2	10	9	10	9	10	9	8^^	8**
Weeks 7 - 8	26	20	31***	20	29***	20	28***	21*

^^ p<0.01 William's test; * p<0.05, ** p<0.01, Shirley's test

F1 Generation – Recovery (g/animal/d): There was no significant difference between food consumption in control and treated animals during the entire 10 week period.

Ophthalmoscopy: F1 Generation – group mean incidences

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Lens suture lines	11/15	12/15	15/15*	14/15	14/15	10/15	13/15	13/15
Hyaloid remnant	11/15	11/15	11/15	10/15	11/15	13/15	12/15	10/15

* p<0.05, Trend test

Electrocardiography: No data.

Hematology:

F1 Generation – Day 21

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
HGB (g/dl)	9.9	10.6	8.3*	8.8***	9.1*	9.6***	9.8	10.1***
PCV (%)	31.3	33.5	26.6	28.5***	29.6	30.9***	31.1	32.0***
MCV (fl)	62.4	65.6	55.5*	58.4**	60.1*	63.4***	60.6*	61.7**
MCH (pg)	19.8	20.8	17.3**	18.0***	18.5**	19.8***	19.0**	19.4**
WBC ($10^3/\mu L$)	5.35	4.94	7.73*	5.90	7.72*	5.73	6.58*	6.51
Lymphocy. ($10^3/\mu L$)	3.95	3.83	5.22*	4.33	5.72*	4.13	4.59*	4.66

* p<0.05, ** p<0.01, *** p<0.001

F1 Generation – Day 70

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
RBC ($10^6/\mu L$)	7.97	7.67	7.82	7.70	7.92	7.55	7.71*	7.42
MCV (fl)	56.1	55.5	55.8	55.3	55.9	55.9	57.6*	56.6*
MCHC (g/dl)	35.1	36.0	34.9	35.9	35.0	35.7*	34.8	35.6*
WBC ($10^3/\mu L$)	14.28	12.12	13.17	12.91	18.25***	14.94**	19.34***	15.35**
Lymphocy. ($10^3/\mu L$)	11.72	10.23	11.06	11.04	15.41***	12.97**	16.05***	12.95**
Monocytes ($10^3/\mu L$)	0.59	0.43	0.57	0.48	0.80*	0.56**	0.98**	0.58*
Basophils ($10^3/\mu L$)	0.04	0.03	0.03	0.03	0.05*	0.04	0.06**	0.04

* p<0.05, ** p<0.01, *** p<0.001

Clinical chemistry:

F1 Generation – Day 21

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
BUN (mg/dl)	22.8	26.1	25.8	25.2	21.7	21.1	30.7*	31.6
ALP (u/L)	679	666	827	667	710	683	960*	993***
Ca (mg/dl)	10.6	10.7	10.7	10.6	10.8	10.9	11.1*	11.1
Phosphorus (mg/dl)	9.1	9.3	9.8*	9.5	9.4*	9.3	10.0***	9.5

* p<0.05, ** p<0.01, *** p<0.001

F1 Generation – Day 70

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Creatinine (mg/dl)	0.6	0.7	0.7	0.7	0.6	0.6**	0.6	0.6**
Glucose (mg/dl)	84	98	86	95	106***	98	117***	117***
ALP (u/L)	337	247	286**	217	277***	233	333*	214
ALT (u/L)	46	35	40	37	45	44***	48	43**
Albumin (g/dl)	3.7	3.8	3.5***	3.6**	3.5***	3.6**	3.6***	3.5***
Globulin (g/dl)	2.8	2.5	2.9	2.7*	2.9	2.7*	2.7	2.7*
A/G ratio	1.3	1.5	1.2	1.4***	1.2	1.3*	1.3	1.3*
T. protein (g/dl)	6.6	6.3	6.3	6.3	6.4	6.3	6.3*	6.2
Cholesterol (mg/dl)	90	86	109	116***	110	120***	89	129***
Triglycerides (mg/dl)	46	43	57*	58	63*	68**	53*	90***
Ca (mg/dl)	10.5	10.4	10.4	10.5	10.8**	10.8*	11.0***	10.7*
Phosphorus (mg/dl)	8.6	7.8	8.8	8.2	8.6	8.7*	8.8	8.3*
Na (mmol/L)	144	142	144	142	143*	141*	143*	140***
K (mmol/L)	3.8	3.5	3.9	3.9**	4.1**	3.9**	4.1**	4.1***

* p<0.05, ** p<0.01, *** p<0.001

Urinalysis: No data.

Organ weights: (g); F1 Generation – Day 70

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Brain		1.69		1.62**		1.65**		1.62**

Heart		0.87		0.91		0.92		0.94*
Heart (%)	0.40	0.42	0.43	0.44	0.42	0.45*	0.44**	0.47**
Liver		8.00		8.70		9.36**		9.93***
Liver (%)	4.19	3.86	4.50**	4.16*	4.62***	4.65***	4.76***	4.93***
Ovaries		0.08		0.08		0.08		0.07*
Kidneys		1.47		1.50		1.63***		1.60***
Kidneys (%)		0.71		0.73		0.82*		0.80*
Thyroid glands		0.014		0.016*		0.015*		0.016*
Thyroid glands (%)	0.006	0.007	0.006	0.008*	0.006	0.008*	0.007**	0.008*
Thymus (%)	0.17		0.22**		0.19**		0.19**	
Testes (%)	1.23		1.27		1.25		1.33*	

Absolute wt (g), Relative wt (to body wt) = %; * p<0.05, ** p<0.01, *** p<0.001

Organ weights: (g); F1 Generation – Recovery: Only organs from males were weighed. There was no significant difference between control and treated absolute or relative organ weights at the end of the recovery period.

Gross pathology: F1 Generation – Day 70

Dose (mg/kg/d)	0		20		60		180	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Epididymides Abnormal size							1/10	
Kidney Abnormal color			2/10		2/10		2/10	
Pelvic dilatation	3/10		1/10		2/10		4/10	
Liver Abnormal color			1/10	2/10		1/10	1/10	1/10
Lungs Abnormal color							1/10	1/10
Abnormal shape							1/10	
Pancreas Abnormal color							1/10	
SM-lymph nodes Abnormal color				1/10	1/10			1/10
Testes Abnormal size							1/10	
Thymus Abnormal color				2/10	1/10	1/10		1/10
Eyes Abnormal color						1/10		1/10
Uterus Distended				3/10				2/10

Histopathology: F1 Generation – Day 70

Dose (mg/kg/d)	0		20		60		180	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Epididymides ↓ sperms							1/10(4)	
Heart Chronic myocarditis	1/10(2)		2/10 1/10(1) 1/10(2)				2/10(1)	
Kidney Hydronephrosis	3/10 2/10(2) 1/10(3)		2/10 1/10(1) 1/10(2)		2/10(2)		5/10 3/10(1) 2/10(2)	
Basophilic tubules	7/10(1)		10/10(2)		10/10 1/10(1) 8/10(2) 1/10(3)		9/10 5/10(1) 4/10(2)	
Liver: Mixed inflammatory cells	1/10(1)		1/10(1)		2/10(1)			

Bile duct proliferation			1/10(1)				1/10(1)	
Granulomatous inflammation							1/10(4)	
Testes:degeneration Germinal epithelium			1/10(1)				1/10(4)	
Brain Diffuse vacuolation				10/10 1/10(1) 5/10(2) 4/10(3)		2/10 1/10(1) 1/10(2)		6/10 2/10(1) 3/10(2) 1/10(3)
Sciatic nerve Vacuolation				10/10 3/10(1) 6/10(2) 1/10(3)		3/10(1)		2/10 1/10(2) 1/10(3)
Tibial nerve Vacuolation				5/10 3/10(1) 2/10(2)		1/10(1)		3/10 2/10(1) 1/10(3)
SM-Lymph node Erythrophagia				2/10 1/10(1) 1/10(4)		2/10 1/10(1) 1/10(2)		1/10(3)
Lymphoid hyperplasia				1/10(2)				
Uterus Distended				5/10 2/10(1) 2/10(2) 1/10(3)				2/10(2)
Polyp-benign tumor								1/10
Vagina Diestrous		5/10		3/10		2/10		2/10
Monoestrous		3/10		1/10		4/10		2/10
Estrous		1/10		2/10		3/10		4/10
Proestrous				4/10				1/10

Histopathology: F1 Generation – Recovery (Male kidneys)

Dose (mg/kg/d)	**0**	**20**	**60**	**180**
Sex	**M**	**M**	**M**	**M**
Kidneys: Basophilic Cortical tubules	4/5 1/5(1) 3/5(2)	5/5 4/5(2) 1/5(3)	5/5 2/5(1) 3/5(2)	5/5 2/5(1) 3/5(2)

1 = minimal, 2 = slight, 3 = moderate, 4 = marked

Toxicokinetics:

Mean plasma concentrations of OGT 918 (ng/ml) in male and female rats following repeated oral administration of OGT 918 at 20, 60 and 180 mg/kg/day

Sampling Day	Dose (mg/kg/day)					
	20		60		180	
	Males	Females	Males	Females	Males	Females
21	2186	2171	5483	7010	15755	14662
35	1632	1583	3227	3317	4783	6199
70	1732	1409	2684	2787	5612	5778

Other: Please note that the F1 generation were not mated. The pregnancy and litter information below was obtained when the F0 dams gave birth.

Pregnancy and litter data – F1 generation: The following pregnancy and litter parameters (number of offspring born per litter, mean live birth index, mean viability index and sex ratio) were similar in all groups.

Pup Development – F1 generation
Group mean litter development observations during lactation – F1generation litters: There were no effects of treatment with OGT 918 on physical development (startle response, pups with open eyes/ears, righting reflex and pupillary light reflex) of the offspring.

E-maze Learning Test – F1 generation (learning & memory retention): There were no effects of treatment with OGT 918 on the learning and memory of male and female offspring compared to control. Run time, correct decisions and 24 hour memory retention was similar in all groups.
Accumulated data for runs 3 to 6, mean time (seconds)

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Session 1-turn to left	6.7±2.4	6.4±2.5	6.5±2.1	6.2±2.5	8.0±3.1	6.3±1.4	7.7±2.7	7.4±2.3
Session 2-turn to right	7.1±2.8	5.8±1.9	7.5±2.8	6.8±1.7	7.0±3.3	6.9±2.5	7.0±2.1	7.6±3.2
Av. # of correct turns-1	5.0	4.9	5.3	4.9	4.9	5.5	5.1	5.2
Av. # of correct turns-2	3.5	4.3	4.3	3.9	4.2	4.0	4.3	3.5
24 hr memory: % turn to left	100	87	80	80	87	80	87	93

Statistically no difference between control and treated groups (Dunnett's test)
Average # of correct turns: 1 = turn to left; 2 = turn to right

Locomotor Activity: Mean Rotarod Test Data (sec) – F1 generation

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
Mean of 5 activities	104 ± 71	99 ± 54	94 ± 55	100 ± 79	87 ± 59	81 ± 63	110 ± 50	58 ± 43

Statistically no difference between control and treated groups (ANOVA)

Auditory Function:
Preyer Reflex: Group mean incicences – F1 generation

Dose (mg/kg/d)	0		20		60		180	
Sex	M	F	M	F	M	F	M	F
% Positive Response	100	100	100	100	100	100	100	100

Group mean sexual development observations – F1 generation

Group	N	Day preputial separation observed	Day vaginal perforation observed
1	15	46.5 ± 1.8	38.6 ± 2.6
2	15	48.0 ± 3.3	41.0 ± 2.3 *
3	15	47.5 ± 3.0	40.0 ± 1.9 *
4	15	49.0 ± 2.0 *	43.5 ± 2.5 ***
Analysis of variance		NS	p<0.001

N = number of animals in mean
* = significantly different from Controls, p<0.05 Williams test
*** = significantly different from Controls, p<0.001 Williams Test

There was a delay in the day balanopreputial separation occurred in males and the onset of vaginal perforation in females in all groups treated with OGT 918; statistical significance was achieved for males receiving 180 mg/kg/day and females receiving 20, 60 or 180 mg/kg/day.

Functional Observation Battery – F1 generation

Home Cage observations: There was no effect of treatment in any group on the home cage observations carried out on either of the test days.

Removal from home cage: No effect of treatment was recorded on removal from home cage on Days 21 or 60.

Open field: There was no effect of treatment on the incidence of observations in the open field on Day 21 or 60.

Other tests: Righting reflex, air righting reflex, vision and hindlimb tactile placing were similar in all groups and showed no effect of treatment with OGT 918.

Sperm analysis:

Group mean analysis of sperm parameters – Day 70

Group number	N	Motility %	Concentration M/ml	VAP µm/s	VSL µm/s
1	15	89 ± 5	16.88 ± 4.53	61.2 ± 4.6	21.7 ± 4.4
2	14	83 ± 10	13.96 ± 4.13*	63.3 ± 6.2	19.5 ± 5.6
3	15	84 ± 6	12.69 ± 3.12**	62.2 ± 4.2	20.6 ± 6.1
4	15	86 ± 6	12.43 ± 4.80**	62.1 ± 5.1	23.2 ± 5.9
Analysis of Variance		NS	p<0.05	NS	NS

N = number of animals in the mean
* = significantly different from Controls, p<0.05 Williams test
** = significantly different to Controls, p<0.01 Williams test

Group 1 = 0 g/kg/d, Group 2 = 20 mg/kg/d, Group 3 = 60 mg/kg/d, Group 4 = 180 mg/kg/d

Group mean sperm morphology – Day 70

Parameter	Group 1	2	3	4	Analysis of variance	Kruskal-Wallis
N	15	14	15	15		
No. normal	196 ± 5	176 ± 13 +++	164 ± 17 +++	163 ± 14 +++	-	p<0.001
No. headless	1.47 ± 2.85	13.71 ± 7.45 +++	21.93 ± 11.99 +++	16.87 ± 10.23 +++	-	p<0.001
No tailless (with mid piece)	0.47 ± 0.74	0.43 ± 0.94	0.13 ± 0.35	0.60 ± 1.12	NS	
No. with reduced hook	0.93 ± 0.80	8.50 ± 5.64 +++	11.73 ± 5.75 +++	17.60 ± 4.82 +++	-	p<0.001
Miscellaneous abnormalities	0.67 ± 1.23	1.86 ± 1.75 *	2.47 ± 2.36 *	1.67 ± 1.40 *	NS	

N = number of animals in mean
+++ = significantly different from Controls, p<0.001 Shirleys test
* = significantly different from Controls, p<0.05 Williams test

Group mean homogenization resistant spermatid count – Day 70

Group number	N	Average number Spermatids/0.1ul	Spermatids/ml x 10^6	Spermatids/Testis x 10^7
1	15	254.3 ± 81.1	2.5 ± 0.8	5.1 ± 1.6
2	15	169.5 ± 32.9 *	1.7 ± 0.3 *	3.4 ± 0.7 *
3	15	228.2 ± 51.9 *	2.3 ± 0.5 *	4.6 ± 1.0 *
4	15	217.1 ± 70.6 *	2.2 ± 0.7 *	4.3 ± 1.4 *
Analysis of Variance		p<0.01	p<0.01	p<0.01

N = number of animals in the mean
* = significantly different from Controls, p<0.05 Williams test

Group mean semen analysis parameters - Recovery

Group number	N	Motility %	Concentration M/ml	VAP µm/s	VSL µm/s
1	5	89 ± 5	14.54 ± 3.65	66.4 ± 2.5	27.3 ± 2.8
2	5	91 ± 4	16.06 ± 1.59	65.0 ± 3.8	29.4 ± 3.4
3	5	91 ± 3	18.96 ± 6.20	67.5 ± 4.6	28.2 ± 6.1
4	5	87 ± 5	16.55 ± 4.16	64.4 ± 3.3	29.5 ± 8.6
Analysis of Variance		NS	NS	NS	-
Kruskal-Wallis					NS

N = number of animals in the mean

Group mean sperm morphology - Recovery

Parameter	Group 1	2	3	4	Analysis of variance
N	5	5	5	5	
No. normal	195 ± 5	196 ± 2	196 ± 1	194 ± 3	NS
No. headless	3.80 ± 4.71	2.40 ± 1.52	1.80 ± 1.48	2.80 ± 0.84	NS
No tailless (with mid piece)	0.40 ± 0.89	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.45	NS
No. with reduced hook	0.40 ± 0.89	1.20 ± 0.84	1.20 ± 2.17	2.60 ± 2.41	NS
Miscellaneous abnormalities	0.00 ± 0.00	0.80 ± 0.84 *	1.40 ± 0.55 **	0.80 ± 0.84 *	p<0.05

NS = not significant
* = significantly different from Controls, p<0.05, Williams Test
** = significantly different from Controls, p<0.01, Williams Test

N = number of animals in mean

Group mean homogenization resistant spermatid count - Recovery

Group number	N	Average number Spermatids/0.1ul	Spermatids/ml x 10^6	Spermatids/Testis x 10^7
1	5	257.6 ± 64.6	2.6 ± 0.6	5.2 ± 1.3
2	5	294.8 ± 53.3	2.9 ± 0.5	5.9 ± 1.1
3	5	325.8 ± 98.6	3.3 ± 1.0	6.5 ± 2.0
4	5	318.0 ± 74.2	3.2 ± 0.7	6.4 ± 1.5
Analysis of Variance		NS	NS	NS

N = number of animals in the mean

Summary of individual study findings:

To investigate the effects of OGT 918 on juvenile development of the rat, OGT 918 was administered to male and female juvenile rats from Day 12 postpartum, up to necropsy on Day 21, 35 or 70 of age and following a recovery period of at least 10 weeks after Day 70 of age.

On Days 12 to 21 post-partum the F1 generation pups were dosed, once daily by oral gavage. From Day 21 onwards the total daily dosage was divided into three equal doses and the animals were dosed three times daily with an interval between doses of at least six hours. A constant dose volume of 10 ml/kg/dose was used. Dose levels were 0, 20, 60 and 180 mg/k/day.

Mortality was observed at all dose levels: 2/80 (0 mg/kg), 1/80 (LD-20 mg/kg), 5/80 (MD-60 mg/kg) and 3/80 (HD-180 mg/kg). The causes of demise of these animals were not disclosed and it is not apparent from the individual histopathology data. Sponsor stated that they were not treatment related. Clinical signs of head tilting (left) was observed in all dose groups including control. The incidence was not dose-dependent and could be due to neurotoxicity. Dose-dependent increase in incidence of distended abdomen was observed at doses ≥ 60 mg/kg/d and it is treatment related. Ridges on whole tail was observed at doses ≥ 20 mg/kg/d. Incidence did not show dose-dependency. Clinical signs observed in animals sacrificed in extremis included hypoactivity, shallow/labored breathing, hunched posture, pale/cold extremities, piloeretion, unsteady gait (1 male – MD), and tremors (1/40 female – MD).

Blood samples taken on Day 70 showed significant decreases in RBC (HD-M) and MCHC (MD & HD-F). WBC, lymphocytes and monocytes were significantly increased in MD and HD animals relative to control. Basophils were slightly but significantly increased in MD and HD males only. There were various treatment related changes in blood chemistry parameters measured. Of these the most significant were the increased cholesterol (all treated females), triglycerides (all treated males and in MD and HD females) and glucose (MD males, HD males and females) concentrations observed on Day 70.

Sperm concentration was significantly decreased in a dose-dependent manner at Day 70. With regards to sperm morphology, number of normal sperms was significantly decreased whereas number of headless sperms was significantly increased in all treated males relative to control. Number of sperms with reduced hook was significantly increased in a dose-dependent manner. Miscellaneous abnormalities were also significantly increased in all treated males relative to

control. Average number of spermatids was also significantly decreased in all treated males relative to control. At the end of the 10 week recovery period, all sperm parameters were similar to those of control except for the significant increase in miscellaneous sperm abnormalities in all treated males relative to control.

OGT 918 had no effect on physical development, learning, locomotor activity, auditory function, righting reflex, air righting reflex, vision and hindlimb tactile placing of the F1 pups. There was no effect of treatment in any group on the home cage, open field and removal from home cage observations. However, evidence of neurotoxicity in females dosed ≥ 20 mg/kg/d, marked (brain, sciatic/tibial nerve) vacuolation was observed. There was a delay in the day balanopreputial separation occurred in males and the onset of vaginal perforation in females in all groups treated with OGT 918; statistical significance was achieved in HD males and all treated females.

The target organs of toxicity included the epididymides (↓ sperms), heart (chronic myocarditis), kidney (hydronephrosis, basophilic tubules), liver (inflammation, bile duct proliferation), testes (degeneration of germinal epithelium), brain, sciatic and tibial nerves (diffuse vacuolation) and submandibular lymph node (erythrophagia, lymphoid hyperplasia). NOAEL could not be established because of decreased sperm concentration, altered sperm morphology, kidney effects (hydronephrosis, basophilic tubules), liver inflammation and chronic myocarditis observed at the LD. moreover, mortality occurred at all dose levels including control and it not clear if this is drug-related or not.

IX. SPECIAL TOXICOLOGY STUDIES:

Study title: Sensitization Potential of SC-48334: Mouse Ear Swelling Test.
Study No: PSA-90S-3555
Rationale: To evaluate the sensitization potential of SC-48334 to provide information for safe handling and manufacturing procedures.
Results: SC-48334 is not a sensitizer at the concentration (30% w/v) tested in this study.

Study title: Exploratory Primary Dermal Irritation Study of SC-48334 In rabbits.
Study No: PSA-89S-3546
Rationale: To evaluate the primary dermal irritation potential of SC-48334 to provide information for safe handling and manufacturing procedures.
Results: The results indicate that SC-48334 was mildly irritating. The positive control was classified as moderately irritating and the negative control was non-irritating.

Study title: The Effect of In Vivo Administration of SC-48334 upon Lymphocyte Subset Frequencies and Functional Lymphocyte Responses.
Study No: BRD-88D-1395
Rationale: To investigate the effect of OGT 918 on immune response and lymphocyte subsets.
Dosing: The drug was administered by IP injection into mice at 30, 100 or 300 mg/kg every 8 hours for 4 to 10 days. These total doses were administered as three equally divided doses/day.
Results: Mice treated with OGT 918 for 4 to 10 days possessed normal levels of IgM and CD8 positive spleen cells. CD3 and CD4 positive cells were increased slightly following 4 or 10 days of OGT 918 administration. The absolute frequency of CD4 positive cells increased 5-7% among mice treated with OGT 918 at 300 mg/kg. Increases among this subset of T cells were dose dependent. This change in CD4 positive cell frequencies, increased with T4:T8 ratios by 35% and was statistically significant following 10 days in vivo administration of OGT 918. Ia positive cells decreased slightly (7%) among mice treated for 10 days. Mitogen responses to T

cell and B cell mitogens were evaluated after 4days of OGT 918 treatment with no significant change in overall responsiveness. Administration of OGT 918 for 10 days, which included injection of T helper cell independent antigen TNP-KLH on Day 4, did not significantly affect serum levels or anti-TNP antibody.
Conclusion: OGT 918 does not significantly alter antigen or mitogen induced lymphocyte responses in vivo or ex vivo, respectively. A significant increase in T4:T8 ratios was observed among mice treated with OGT 918. Other lymphocyte subsets were quantified and no significant alterations in frequencies of IgM, Ia, or CD3 positive cells were observed.

Study Title: Lymphocyte Subset Analysis in Peripheral Blood and Spleen of Cynomolgus Monkeys Exposed to SC-48334 During a 4-Week Intravenous Infusion Toxicity Study (SA4144).
Study No.: P3096039
Rationale: To determine the effects of OGT 918 on lymphocyte subsets in the cynomolgus monkey as an index of the immunotoxic potential of this compound.
Dosing: Doses of 60, 300 and 600mg/kg/day were administered IV for 4 weeks. These total doses were administered as three equally divided doses/day. The lymphocyte subsets analyzed in this study included, but were not limited to, total B cells (CD20+CD2-cells), total T cells (CD2+CD20-cells), CD4+helper T cells (CD2+CD4+) and CD8+T suppressor/cytotoxic cells.
Results: IV infusion of OGT 918 at doses of 60, 300 and 600 mg/kg/day for 4 weeks did not result in any significant alterations in peripheral blood lymphocyte subsets in either male or female monkeys. Similarly, OGT 918 was not found to have any significant effects on lymphocyte subsets in the spleens of male monkeys. In female monkeys, all splenic lymphocyte parameters, with the exception of a decrease in the number of CD8+HLA-DR+ cells, were also found to be unaffected by the OGT 918 treatment. Since CD8+HLA-DR+ cells have been defined as an immature T cell subset, the decrease in CD8+HLA-DR+ cells is likely to be due to an indirect effect of this compound on thymus cell maturation in the females.
Conclusion: IV administration of OGT 918 for up to 4 weeks has no direct cytotoxic effect on lymphocyte subsets in the peripheral blood or spleen of cynomolgus monkeys.

Study Title: Lymphocyte Subset Analysis in Peripheral Blood, Spleen and Thymus of Rats Exposed to SC-49483 During a 26 Week Oral Safety Study (SA4085).
Study No.: PSA-96S-30-960058
Rationale: To determine the effects of OGT 924 on the lymphocyte subsets in the rat as an index of the immunotoxic potential of this compound.
Dosing: Animals were dosed for 26 week with OGT 924 at doses of 300, 600 and 1200 mg/kg/day. These total doses were administered as three equally divided doses/day. A pair-fed control group was included to control for any potential effects that a lowered dietary intake would have on lymphocyte subsets in the OGT 924 treated animals. The lymphocyte subsets analyzed included, but were not limited to, total B cells (CD45RA+ and/or IgM+cells), total Tcells (Pan T+or CD3+cells), CD4+ helper T cells (CD3CD4+) and CD8+Tsuppressor/cytotoxic cells (CD3+CD8+). Additionally, organ specific lymphocyte subsets, including the immature CD4+CD8+ (double positive) T cells of the thymus, were assessed as part of these analyses.
Results: OGT 924, when administered to rats at doses 300, 600 and 1200mg/kg/day, was not cytotoxic to mature immunocompetent and immature thymic lymphocytes. An increase in the CD4:CD8 T cell ratios of the blood and spleen was observed in OGT 924 exposed rats. This increase in the CD4:CD8 ratio resulted from a statistically significant increase in the absolute number of CD3+CD4+ T cells in the blood and non-significant increases in the absolute numbers of CD3+CD4+ and decreases in the absolute numbers of CD3+CD8+ T cells, respectively, of the spleen.

Conclusion: OGT 924 does not have a direct cytotoxic effect on rat lymphocytes. However, the changes in the CD4:CD8 ratios observed in the OGT 924 treated animals may indicate a slight alteration in lymphocyte trafficking induced by this compound.

Study Title: Twelve-Day Exploratory Study of the Gastrointestinal Effects of SC-48334 and 65% Dietary Dextrose in the Female Rat.
Study No.: PSA-89S-3482
Rationale: To determine if a dextrose diet would decrease the incidence of diarrhea previously exhibited in animals dosed with OGT 918.
Dosing: OGT 918 was administered by oral gavage for 12 days at 0 mg/kg and 1680 mg/kg/day.) The total dose was administered as three equally divided doses/day.
Animals received ——— purified diet with 65% dextrose and one group (Positive Control – 1680 mg/kg/day) received ——— Rodent Chow.
Results: The ——— purified diet with 65% dextrose prevented the development of diarrhea induced by a daily dosage of 1680 mg/kg OGT 918 in female rats for a period of 12 days.

Study Title: Two-Week Exploratory Study of the Gastrointestinal Effects of SC-48334 and 65% Dietary Dextrose in the Female Rat.
Study No.: PSA-89S-3475
Rationale: To determine if a dextrose diet would decrease the incidence of diarrhea previously exhibited in animals dosed with OGT 918.
Dosing: OGT 918 was administered by oral garage for 5 days to ——— CD female rats (9-10/group) at 2400 and 3200 mg/kg/d. These total doses were administered as three equally divided doses/day. The positive control group (2400 mg/kg/day OGT 918) was fed ——— Rodent Chow while the three remaining groups received ——— diet with 65% dextrose (0, 2400 and 3200 mg/kg/day OGT 918). After Study Day 5, dosing was discontinued due to the declining general health of the animals. Animals were observed until sacrifice on Study Day 12.
Results: 3/10 2400 mg/kg/day and 7/10 3200 mg/kg/day animals fed the diet with 65% dextrose were found dead or killed in extremis. No control (dextrose diet only) or positive control (2400 mg/kg/day OGT918, ——— diet) animals died. Diarrhea was observed in positive control animals during the first day of dosing and in the 2400 and 3200 mg/kg/day animals fed the dextrose diet by the third. Swollen limbs and/or face was observed in OGT 918 treated animals on the first day of dosing regardless of diet.

Bodyweight, bodyweight gain and food consumption were decreased for all OGT 918 treated groups during the five day dosing period. All groups had comparable weight gains and food consumption by the end of the observation period. Bodyweights remained decreased for the duration of the study.

Necropsy of the animals found dead or killed in extremis revealed watery or bloody intestinal content in 7 animals.

In was concluded that the ——— purified diet with 65% dextrose did not prevent the development of diarrhea induced by a daily dose of either 2400 or 3200 mg/kg/day OGT 918. Despite the delayed onset of diarrhea, decreased weight gain attributable to the dextrose diet (seen during the acclimation period) may have contributed to the increased mortality exhibited in the groups receiving both OGT 918 and the dextrose diet.

Study Title: Two-Week Exploratory Oral Dose (T.I.D.) Study of Gastrointestinal Effects of SC-48334 in the Female Rat.
Study No.: PSA-89C-3408.
Rationale: To investigate the effects of different dosing vehicles (glucose solution, HCL solution or distilled water) on the GI changes observed in rats given large doses of SC-48334.
Dosing: OGT 918 was administered by oral gavage for either 10 or 11 days to female Sprague-Dawley rats at doses of 2400, 3200 or 3600 mg/kg/day. These total doses were administered as three equally divided doses/day. Animals were divided into 12 groups (10/group). Four groups received distilled water, four an equimolar solution of glucose, and the remaining four an equimolar solution of hydrochloric acid as the dosing vehicle. Control groups in each set received the appropriate vehicle while the remaining three groups were dosed three times daily with either 2400, 3200 or 3600 mg/kg/day OGT 918. Due to the severity of the toxicity observed, half of the animals receiving OGT 918 were sacrificed on Day 12. Dosing was discontinued for the remaining animals until sacrifice on Day 15.
Results: Deaths occurred in all groups receiving OGT 918. The majority of these deaths were observed between Days 5 and 10 with one mortality occurring 24 hours after the final dose.

All animals receiving OGT 918 exhibited persistent diarrhea, distended abdomen, perineal staining, decreased grooming, piloerection and reddened foci on the hind paws. The onset and severity of these findings was dose-dependent and increased with prolonged dosing. There was no apparent difference in the onset of severity of clinical signs between the three different vehicle sets. All clinical findings rapidly diminished following termination of test article administration.

Bodyweight and food consumption were decreased in all groups receiving OGT 918. Both bodyweights and food consumption increased in all OGT 918 treated groups following completion of the dosing regimen.

At necropsy, all rats administered OGT 918 exhibited distended fluid filled ceca regardless of the vehicle employed. Other findings in the cecum indicated the presence of gas in the lumen and occasional hemorrhage of the mucosal surface. Sponsor stated that although many of the reversal animals exhibited slightly to moderately stretched ceca, following the four day recovery period, in general the contents had returned to normal and overall cecal appearance was greatly improved.

It was concluded that no significant beneficial effects were observed as a result of substituting either equimolar glucose or equimolar hydrochloric acid for distilled water as the dosing vehicle.

Study Title: Exploratory Study on the Effects of High Dietary Dextrose on SC-48334 Gastrointestinal Effects in Beagle Dogs.
Study No.: PSA-90S-3552
Rationale: The purpose of this study was to determine if a 63% dextrose diet would modify the gastrointestinal toxicity of OGT 918 in the Beagle dog.
Dosing: OGT 918 was administered orally in gelatin capsules to Beagle dogs. Animals were dosed at 35 mg/kg of OGT 918 three times a day at approximate eight hour intervals (total dose of 105 mg/kg/day) for 15 days. Group 1 animals (2/sex) received the standard diet [redacted] and the Group 2 animals (2/sex) received a modified high dextrose diet [redacted]
Results: There were no differences between groups with regard to bodyweight changes and food consumption. Dogs in both groups lost weight, ate less than during the pre-treatment, and

displayed signs of gastrointestinal (GI) toxicity (vomiting and/or alterations in stool). Signs of GI toxicity were less frequently observed in the dogs receiving the modified high dextrose diet.

Sponsor stated that there were no major differences between the gross lesions of the two groups. Both groups had one animal with a normal intestine. The other animals in both groups had hyperemia of the gastrointestinal tract of variable severity and distribution. Several animals had blood-tinged contents in the ileum and large intestine. The underlying tissue looked hemorrhagic in some animals. Animals in both groups had small thymuses.

Histologically, the lesions also varied among animals but were essentially the same between groups. Changes included hyperemia or vascular congestion, minor focal erosions of the cecal or colonic epithelium and loss of epithelium from villous tips in the small intestine. The exposed lamina propria of these villous tips had an indistinct appearance caused by infiltration with a basophilic amorphous material that may have been mucous. This appearance may have represented post-mortem change rather than a lesion. Gut associated lymphoid tissue (Peyer's patches) and thymic tissue were involuted in both groups.

Based on the pathological findings and other evidence, sponsor concluded that the differences in the incidences of gastrointestinal signs between the two groups were probably not of toxicologic importance. A highly purified diet, (high dextrose, low in complex carbohydrates) did not afford dogs protection against the gastrointestinal effects of OGT 918.

Summary of individual study findings: The effects of in vivo administration of OGT 918 upon lymphocyte subset and frequencies and functional lymphocyte responses were investigated in mice, cynomolgus monkey and rats to determine the immunotoxic potential of the drug. The results indicate that administration of OGT918 at doses of 30, 100 or 300 mg/kg/d for 10 days, which included injection of T helper cell independent antigen TNP-KLH on Day 4, did not significantly affect serum levels or anti-TNP antibody in mice. IV infusion of OGT 918 at doses of 60, 300 and 600 mg/kg/day for 4 weeks did not result in any significant alterations in peripheral blood lymphocyte subsets in either male or female monkeys. Thus IV administration of OGT 918 for up to 4 weeks had no direct cytotoxic effect on lymphocyte subsets in the peripheral blood or spleen of cynomolgus monkeys. Administration of OGT 924 to rats at doses 300, 600 and 1200mg/kg/day, was not cytotoxic to mature immunocompetent and immature thymic lymphocytes of rats. An increase in the CD4:CD8 T cell ratios of the blood and spleen was observed in OGT 924 exposed rats. This increase in the CD4:CD8 ratio resulted from a statistically significant increase in the absolute number of CD3+CD4+ T cells in the blood and non-significant increases in the absolute numbers of CD3+CD4+ and decreases in the absolute numbers of CD3+CD8+T cells, respectively, of the spleen. It was concluded from this investigation that OGT 924 does not have a direct cytotoxic effect on rat lymphocytes. However, the changes in the CD4:CD8 ratios observed in the OGT 924 treated animals may indicate a slight alteration in lymphocyte trafficking induced by this compound.

To determine if a dextrose diet would decrease the incidence of diarrhea previously exhibited in animals dosed with OGT 918, rats were dosed with doses ranging from 1680 mg/kg/d to 3200 mg/kg/d for 12 to 14 days. The animals were also given ——— purified diet with 65% dextrose. The studies demonstrated that ——— purified diet with 65% dextrose prevented the development of diarrhea induced by a daily dosage of 1680 mg/kg OGT 918. At doses of 2400, 3200 and 3600 mg/kg/d, ——— purified diet with 65% dextrose did not prevent the development of diarrhea. Rats were also dosed at 2400, 3200 and 3600 mg/kg/d, using different vehicles (glucose solution, HCL solution or distilled water) to investigate their effect on the GI

changes observed in rats. No beneficial effects were observed as a result of substituting either glucose or HCL solution for distilled water as the dosing vehicle.

Dogs were dosed with OGT 918 at 35 mg/kg/d TID (total of 105 mg/kg/d) gelatin capsules and given a 63% dextrose diet for 15 days to determine if the dextrose diet would modify the GI toxicity of OGT 918 observed in Beagle dogs. The results indicate that the dextrose diet did not afford the dogs protection against the GI effects of OGT 918.

Conclusions: It was concluded from these studies that OGT 918 (mouse, monkey) or OGT 924 (rats) does not have a direct cytotoxic effect on lymphocytes of the various animal species tested. However studies in rat and monkey evaluating lymphocyte subsets indicates a potential for altering lymphocyte trafficking.

Rats dosed up to 1680 mg/kg/d with a 65% dextrose diet prevented the development of diarrhea induced by OGT 918. At doses of 2400, 3200 and 3600 mg/kg/d, the 65% dextrose diet did not prevent the development of diarrhea. No beneficial effects on GI changes were observed as a result of substituting either glucose or HCL solution for distilled water as the dosing vehicle.

Dogs dosed with OGT 918 at 35 mg/kg/d TID (total of 105 mg/kg/d) gelatin capsules and given a 63% dextrose diet for 15 days were not protected against the GI effects of OGT 918.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

General Toxicology Issues:
OGT 918 has been assessed by comprehensive toxicology studies in mice, rats, dogs and primates. The dominant feature of toxicity was profound diarrhea and the effects on the GI tract especially in dogs and rats. By inhibiting glucosylceramide synthase, OGT 918 may affect the activity of other glucosidase which prevent hydrolysis of dissacharides potentially leading to osmotic diarrhea. Weight loss and/or decreased body weight gain as well as signals suggestive of neurotoxicity were observed across species. These effects may relate to accumulation of ceramide which has been associated with signal transduction and apoptosis.

Neurotoxicity:
Clinical signs and histopathology findings suggestive of neurotoxicity were observed in the dog, rat and monkey. Ataxia, diminished/absent pupillary, palpebral or patellar reflexes were observed in the dog at doses ≥ 54X the therapeutic dose based on mg/m^2. In addition, tremor and absent corneal reflexes were also observed at doses ≥ 11X the therapeutic dose based on mg/m^2. However, there was no histopathology finding to support the clinical signs observed in the dog. Histopathology findings in monkey brain (vascular mineralization, mineralization and necrosis of white matter) and spinal cord (vascular mineralization) were observed at doses ≥ 4X the therapeutic dose based on AUC with no apparent clinical signs. Vacuolation of the white matter of the brain was observed in both treated and control rats at doses ≥ 6X the therapeutic dose based on mg/m^2. These findings were not dose-related and since control rats also had these lesions, it is not clear if it is drug related or not. Except for the lesions in the monkey brain and spinal cord, the doses at which clinical signs and histopathology findings of neurotoxicity occurred provide wide safety margins. However, neurotoxicity has been observed at the therapeutic dose in clinical trials. Weanling rats dosed postnatal days 12-70 at ≥ 20 mg/kg/d showed vacuolation of brain and sciatic/tibial nerves. Sexual development appeared slightly delayed (vaginal perforation) but locomotor, learning, righting reflex and cage behavior were unremarkable.

GI Toxicity:
The dominant feature of toxicity was profound diarrhea and GI tract inflammation and necrosis especially in dogs and rats. Severity of the diarrhea increased with dose and sometimes progressed to bloody diarrhea or black tarry stool. In the chronic rat toxicology study, the frequency of diarrhea was high during the early treatment period but completely subsided with time. In the dog, necrosis of crypts of epithelium with dilatation and plugging, necrosis of villous tips, mucosal erosion, congestion and inflammation occurred at ≥ 8X the therapeutic dose based on mg/m^2. GI toxicity in rats occurred at ≥ 7X the therapeutic dose based on AUC. The GI lesions observed include increase in the mitotic figures in (cecal epithelium), ulcer, hyperkeratosis, inflammation, hemorrhage, cytoplasmic vacuolation of chief cells (stomach), dilatation of crypts, necrosis, edema, inflammation (colon), mucosal necrosis, inflammation, hemorrhage (cecum), depletion of goblet cells (intestines), villous atrophy (jejunum and ileum), and serosal fibrosis (esophagus).

While the Rhesus monkey showed GI toxicity similar to those observed in rats and dogs, the cynomolgus monkey had minimal GI lesions in the gut mucosa after doses up to 9X the therapeutic dose (based on AUC) for 1 year with OGT 924. GI lesions in the rhesus monkey (ulcer, inflammation, necrosis and hemorrhage of the colon, cecum) were observed at ≥ 32X the therapeutic dose based on mg/m^2. In the cynomolgus monkey, GI lesions were observed in the cecum, colon, stomach (pigmented macrophages, granulomatous inflammation) at doses ≥ 6X the therapeutic dose based on AUC.

Body Weight Effects:
Decreased mean body weight and/or body weight gain was observed across all species. Mean body weight was decreased by 12% (at doses ≥ 2X the therapeutic dose based on AUC) to 32% (at 10X the therapeutic dose based on AUC) in male rats following 13 to 52 weeks of treatment. Values for female rats range from 14% (at doses ≥ 7X the therapeutic dose based on AUC) to 26% (at 11X the therapeutic dose based on AUC). In mice, mean body weight was decreased by 12% (at 39X the therapeutic dose based on mg/m^2) and diarrhea occurred at doses ≥ 58X the therapeutic dose based on mg/m^2 following 2 weeks of treatment. Diarrhea occurred in all dogs treated with OGT 918 (at doses ≥ 2X the therapeutic dose based on mg/m^2) and to a negligible extent in dogs treated with OGT 924. Mean body weight decrement of 26% was observed in dogs at 89X the therapeutic dose based on mg/m^2. Monkeys treated for 52 weeks with OGT 918 also experienced diarrhea at doses ≥ 11X the therapeutic dose based on AUC. Decreased mean body weight ranging from 18% to 32% for males (at doses ≥ 9X the therapeutic dose based on AUC) and 14% to 26% for females (at doses ≥ 7X the therapeutic dose based on AUC) was observed. The GI toxicity, body weight and diarrhea effects appear to be related. The GI toxicity may have resulted in poor nutrient absorption, diarrhea and consequently decreased body weight and/or body weight gain. The diarrhea could also relate to the pharmacologic activity (inhibition of glucosidase - prevention of hydrolysis of disaccharides, potentially leading to osmotic diarrhea) of the drug.

Lymphoid Toxicity:
Lymphoid/lymphocyte depletion was observed in the spleen, thymus, mesenteric lymph node and submaxillary lymph node in rats dogs and mice. In rats, lymphocyte depletion occurred at doses ≥ 3X the therapeutic dose based on AUC and at ≥ 55X the therapeutic dose based on mg/m^2 following 4 weeks of treatment. Chronic studies in the rat revealed, lymphoid atrophy of the spleen, mesenteric lymph node and thymus at doses ≥ 4X the therapeutic dose based on AUC. Treatment up to 4 weeks in the dog caused thymic involution (4X - mg/m^2), decreased thymocytes, atrophy (lymphoid depletion) of the Peyer's patches of the ileum (9X - mg/m^2) and

lymphoid depletion of the mesenteric lymph node (≥ 4X – mg/m^2). Thymic involution was observed in the mouse at doses ≥ 20X the therapeutic dose based on mg/m^2).

Bone marrow toxicity:
Bone marrow toxicity was observed in subacute rat studies and chronic monkey studies. In rats, bone marrow hypocellularity with fat replacement occurred at doses ≥ 3X the therapeutic dose based on AUC. Bone marrow hypocellularity with necrosis was also observed in the rats at 136X based on mg/m^2. Chronic monkey studies also revealed bone marrow hypocellularity with fat replacement at 9X the therapeutic dose based on AUC. Bone marrow toxicity has been observed at the therapeutic dose in clinical trials.

Effects on RBC Parameters:
Decreased red blood cell parameters were observed in the mouse, rat, dog and monkeys. Hemoglobin, hematocrit and RBC count were all significantly decreased in the dog at 8x the therapeutic dose based on mg/m^2. In rats, hemoglobin, hematocrit and RBC were also significantly decreased at doses: ≥ 10X the therapeutic dose (AUC, 4 weeks), ≥ 2X the therapeutic dose (AUC, 13 weeks), ≥ 5X the therapeutic dose (AUC, 26 weeks) and ≥ 4X the therapeutic dose (AUC, 52 weeks). Monkeys also showed decreased RBC, hematocrit and hemoglobin at 8X the therapeutic dose (AUC, 52 weeks). While similar effects were observed in the mouse and the decrements were dose-dependent, the difference between treated and control groups was not statistically significant. The decreased hematology parameters may be secondary to the bone marrow toxicity or secondary to liver/kidney pathology induced by the pharmacologic activity of the drug.

Reproductive Toxicity:

In the male rat fertility studies, OGT 918 affected normal morphology (headless sperms, sperms with reduced hooks & miscellaneous abnormalities) and motility (VAP) of sperm (≥ 20 mg/kg/d), which was consistent with the observed reduction in fertility. Reversibility of these parameters was demonstrated on cessation of treatment (6 or 13 week recovery). In further studies on male fertility, rats were dosed and mated with untreated females. The effects on male fertility and sperm parameters were confirmed. Treatment resulted in an increase in the number of unfertilised and fragmented ova, but those that were fertilised proceeded to develop normally. When males were mated after a 6-week treatment-free period, pregnancy parameters were within normal ranges. Sperm morphology and motility was similar to that of control animals after a 13-week treatment-free period. NOAEL could not be established because of the decreased sperm concentration, decreased number of normal sperms, increased headless sperms and increased sperms with reduced hooks in LD males. However, the LD tested is 0.6x the human dose based on mg/m^2.

When OGT 918 was administered to female rats prior to mating through GD 17, fetotoxic effects were observed at exposures associated with maternal toxicity. The NOAEL for maternal toxicity was 60 mg/kg/day (2X human exposure based on mg/m2) based on a statistically significant decrease in body weight (10%) and body weight gain (-29%) at 180 mg/kg/day. Post-implantation loss was observed at doses ≥ 60 mg/kg/day. The NOAEL for fetal developmental toxicity was 20 mg/kg/day (<1X human exposure based on mg/m^2) based on a significant increase in early embryo fetal deaths at ≥ 60 mg/kg/day (24 vs. 10 in control). Several fetal findings are observed associated with maternally toxic exposures (>60 mg/kg/day). Increased placental weight, in addition to the following visceral malformations: absent innominate artery, misshapen ventricles, reduced lung size, increased kidney pelvic cavitation and dilated ureters are found in fetuses from dams given 180 mg/kg/day. Only the absent innominate artery

incidence exceeds control and historical control mean and range at 180 mg/kg/day; at a dose associated with matemal toxicity. Decreased fetal weight was observed at 180 mg/kg/day. Several skeletal malformations are observed at doses ≥ 60 mg/kg/day including: wavy ribs, malformed scapula, misshapen sternum, misshapen vertebrae most of these findings are within the historical control range. Skeletal variations consisting of incomplete ossification of: occipital, thoracic vertebra, sternebra (1-6), pubis, metacarpals (forelimb), phalanges (forelimb) and metatarsals (fore and hind limb) bones is observed at doses ≥ 60 mg/kg/day. However the incidences of these findings may exceed control and historical mean but are within the historical control range.

In rabbits dosed with 15, 30, 45 mg/kg/day during organogenesis (GD 6-18) maternal toxicity was evident at 15 mg/kg/day with a -50% decrease in body weight gain and mortality (2/20 GD 22-23) at 30 mg/kg/day. It is unclear if the premature deaths were specifically related to parturition as in the rat although they were considered treatment related. Macroscopic exam of those dams consisted of dark mesenteric lymph nodes and abnormal red stomach on GD 22 and 23. Statistically significant, dose dependent increase in additional aortic arch blood vessels at all dose levels and incomplete ossification of maxilla, hyoid and forelimb epiphyses (not ossified) at 15 mg/kg/day. The fetal findings at 15 mg/kg/day (<1X human therapeutic exposure) occur at exposures associated with maternal toxicity. An earlier rabbit Segment II study using 3, 10, 30 mg/kg/day during GD 6-18 conducted by Searle (the sponsor at the time) did not show maternal mortality although body weight gain decreased by 33% at 30 mg/kg/day. The fetal/litter incidence of missing brachiocephalic artery increased with dose but remained below the historical control mean (range not provided). The presence of a left subclavian branching variation increased by fetal incidence at ≥ 10 mg/kg/day but remained below historical control fetal/litter mean incidence. Skeletal variations including: misshapen thoracic centrum, unossified medial phalange and pubis were increased at 30 mg/kg/day whereas incomplete ossification of the hyoid centrum occurred dose dependently at 10 mg/kg/day and incomplete sternebra at 3 mg/kg/day (not dose dependently). Historical control data is not provided, however the findings occur in general at doses associated with maternal toxicity and the incomplete ossification which occur at lower doses are consistent with the previous study.

In a Segment III study where rats were given 20, 60, 180 mg/kg/day during organogenesis through lactation (GD6- PPD 20) dystocia and delayed parturition were observed. Three females were sacrificed in extremis Gestation Day 22, 25, 26, 1/25 in MD and 2/25 in HD groups as a function of deteriorating clinical condition associated with extended parturition. None had surviving pups. A 35% decrease in body weight gain was observed at the HD. An increased gestation duration (22.7 vs. 21.9 days control), a 48% decrease in births and 25% decrease in live births were observed at HD. At >60 mg/kg/day the mean cumulative pup survival index decreases dose dependently by 12-19% and pup body weight gain decreases dose dependently by 6-18%. Drug treated groups had increased numbers of dead or sacrificed in extremis pups by 159% at >20 mg/kg/day. Most of these animals were found with uninflated lungs and absence of milk in the stomach at necropsy. Locomotor co-ordination assessed by mean rotarod test data (n=5 runs), shows a significant decrease in HD males. The sponsor attributes this to decreased bodyweight. However pup body weights are similarly reduced in females and MD rats which did not show adverse effects in locomotor co-ordination. A statistically significant delay (2-3 days) in the onset of vaginal perforation in pups from groups dosed ≥ 60 mg/kg/day. Mating and fertility parameters of the crossed FI generation were unremarkable.

Cardiac toxicity:

Cardiac toxicity, characterized as degenerative cardiomyopathy was observed in rats at doses ≥ 22X the therapeutic dose based on AUC, following a 13-week study. This lesion was not

observed at the end of the recovery period. In a 52-week study, degenerative cardiomyopathy was observed again in rats at doses ≥ 4X the therapeutic dose based on AUC. However, some control animals also had the lesion. Very little/no recovery occurred after a 4-week period. Acute inflammation of the heart was noted in monkeys found dead at doses ≥ 32X the therapeutic dose based on mg/m^2 after 4 weeks of treatment. The sponsor tries to suggest that degenerative cardiomyopathy is age-related. However, the incidence/severity of degenerative cardiomyopathy increased with drug treatment in males and is present at 13 weeks where the rats are clearly not aged.

Renal Toxicity:
Chronic progressive nephropathy was observed in rats at 22X the therapeutic dose based on AUC, following a 13-week study. In 26-week and 52-week studies, the same lesion was observed at 4x and 3x the therapeutic dose based on AUC, respectively. Very little/no recovery occurred after a 4-week period.

Ocular Toxicity:
Cataracts were observed in rats after 52-week studies at 4x the therapeutic dose based on AUC. Partial recovery was observed after a 4-week period. The cataracts may be related to the pharmacologic effects of the drug. The drug causes perturbations in lipid metabolism. Perturbations in lipid metabolism are associated with enhanced lipid peroxidation which generates oxygen free radicals. Oxygen free radicals are important known causes of tissue damage including cataracts.

APPEARS THIS WAY
ON ORIGINAL

TOXICITIES OBSERVED

DIARRHEA

SPECIES	STUDY DESIGN	TOXICITY	AUC (ng.h/ml)	mg/m²	MULTIPLE OF THERAPEUTIC DOSE (100 mg TID)	
					AUC (ng.h/ml)	mg/m²
Rat	OGT 918: 90, 180, 420, 840 mg/kg/d, 13 weeks.	Diarrhea at ≥ 180 mg/kg/d.	30,350 (M) 60,990 (F)		3X 7X	
	OGT 918: 180, 420, 840, 1680 mg/kg/d, 52 weeks.	Diarrhea at ≥ 840 mg/kg/d	92,135 M + F		10X	
Dog	OGT 918: 20, 40, 80 mg/kg/d, 2 weeks	Diarrhea at ≥ 20 mg/kg/d.		400		≥ 7X
Monkey	OGT 918: 240, 1200, 2400 mg/kg/d, 2 weeks	Diarrhea at ≥1200		14,400		≥ 78x
Mouse	OGT 918: 240, 1200, 2400 mg/kg/d.	Diarrhea at doses ≥ 1200 mg/kg/d. No NOAEL.		3,600		≥ 20X

GI TOXICITY

SPECIES	STUDY DESIGN	TOXICITY	AUC (ng.h/ml)	mg/m²	MULTIPLE OF THERAPEUTIC DOSE (100 mg TID)	
					AUC (ng.h/ml)	mg/m²
Dog	OGT 918: 85, 165, 495, 825 mg/kg/d Oral (capsule) 2 weeks	Necrosis of crypts of epithelium with dilatatio -n and plugging, necros -is of villous tips (≥ 85 mg/kg/d).		LD = 1700		≥ 9X
Dog	OGT 918: 35, 70, 105, 140 mg/kg /d. Dose escalati -on study for 4 weeks.	Small & large intestine (congestion of mucosa, inflammation, necrosis of villous tips, mucosal erosion at ≥ 70 mg/kg/d).		MD = 1400		≥ 8X
Rat	OGT 918: 420, 1680 mg/kg/d, 4 weeks	↑ mitotic figures in cecal epithelium, villous atrophy in small intestines at HD.		HD = 10,080		55X
Rat	OGT 924: 330, 1020, 3670 mg/kg/d, 4 weeks	Stomach (chief cell vacuolation in glandular stomach at ≥ 1020 mg/kg/d)	MD = 92,000		≥ 10X	
Rat	OGT 918: 180, 840, 4200 mg/kg /d, 4 weeks	↑ mitotic figures – cecal epithelium, stomach – hemorrhage, depletion of goblet cells – entire intestines, villous atroph -y - jejunum, ileum at doses ≥ 840 mg/kg/d.		MD = 5,040		≥ 27X

Rat	OGT 924: 300, 600, 1200 Oral, 26 weeks	Esophagus (serosal fibrosis), stomach (cyto –plasmic vacuolation of chief cells), large intesti –ne (mucosal necrosis) at HD.	HD=65,350		7X	
Rat	OGT 918: 180, 420, 840, 1680 Oral, 52 weeks	Enteropathy at HD		HD=10,080		55X
Monkey	OGT 918: 165, 495, 1650, Oral, 4 weeks	Ulcer, inflammation, necrosis, hemorrhage – colon & cecum at ≥ 495 mg/kg/d.		MD = 5,940		≥ 32x
Monkey	OGT 924: 750, 2000 mg/kg/d, 52 weeks.	Cecum, colon, (pigment -ed macrophages – HD males, Stomach – granulomato -us inflammation – LD & HD females)	HD=57,800 LD=52,800 HD=79,900		7X – M 6X – F 9X – F	

WEIGHT LOSS

SPECIES	STUDY DESIGN	TOXICITY	AUC (ng.h/ml)	mg/m²	MULTIPLE OF THERAPEUTIC DOSE (100 mg TID)	
					AUC (ng.h/ml)	mg/m²
Rat	OGT 918: 90, 180, 420, 840 mg/kg/d, 13 weeks.	↓ body wt. gain –11% (HMD males), 31% (HD males), 19% (HD females)	HMD: 88,400 (M) HD: 194,170(M) 140,980 (F)		10X 22X 16X	
	OGT 918: 20, 60, 180 mg/kg/d, 13 weeks.	12% ↓ body wt. gain at HD.	19,600 2,700		2X 0.3X	
	OGT 924: 300, 600, 1200 mg/kg /d, 26 weeks	↓ body wt. 12% (MD males), 23% (HD males).	MD:55,900 HD:68,000		6X 8X	
	OGT 918: 180, 420, 840, 1680 mg/kg/d, 52 weeks.	↓ body wt. 14% (MD-F), 18% (MD-M), 32% (HMD-M), 26% (HMD-F).	MD: 76,750 (M) 56,140 (F) HMD: 84,650 (M) 99,620 (F)		9X 6X 10X 11X	
Dog	OGT 918: 20, 40, 80 mg/kg/d, 2 weeks	10% ↓ body wt. ≥ 80 mg/kg/d.		1600		≥ 9X
Monkey	OGT 918: 165, 495, 1650 mg/kg/d, 4 weeks	↓ body wt. ≥ 165 mg/kg/d.		1,980		≥ 11X
Mouse	OGT 918: 240, 1200, 2400 mg/kg/d, 2 weeks	12% ↓ body wt. at 2400 mg/kg/d.		7200		39x

In rats, decreased body wt./body wt. gain occurred at doses ≥ 180 mg/kg/d = 2X the therapeutic dose.